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(54) Title: METHODS FOR DIAGNOSIS AND THERAPY OF AUTOIMMUNE DISEASE, SUCH AS INSULIN DEPENDENT DIABETES MELLITUS, INVOLVING RETROVIRAL SUPERANTIGENS		
(57) Abstract The invention relates to a process for the diagnosis of a human autoimmune disease, including presymptomatic diagnosis, said human autoimmune disease being associated with human endogenous retrovirus (HERV) having Superantigen (SAg) activity, comprising specifically detecting in a biological sample of human origin at least one of the following: (I) the mRNA of an expressed human endogenous retrovirus having Superantigen (SAg) activity, or fragments of such expressed retroviral mRNA, said retrovirus being associated with a given autoimmune disease, or (II) protein or peptide expressed by said retrovirus, or (III) antibodies specific to the protein expressed by said endogenous retrovirus, or (IV) SAg activity specifically associated with said endogenous retrovirus, detection of any of the species (I) to (IV) indicating presence of autoimmune disease or imminent onset of autoimmune disease.		

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**Methods for Diagnosis and Therapy of Autoimmune
Disease, such as Insulin Dependent Diabetes Mellitus,
involving Retroviral Superantigens.**

The present invention relates to methods for the diagnosis of human autoimmune disease, for example Insulin Dependent Diabetes Mellitus (IDDM), and to methods for identifying substances which can be used in the therapy and prevention of such diseases. The invention further relates to novel human retroviruses involved in autoimmune disease and having superantigen activity, as well as to their expression products.

For some autoimmune diseases such as IDDM, Multiple Sclerosis, arthritis and others, it is known that a combination of genetic, environmental and possibly exogenous infectious factors may be important in precipitating disease. However, the precise roles of each of these factors remains incompletely elucidated. For example, for IDDM, the Major Histocompatibility Complex (MHC) Class II genotype is one of the strongest genetic factors determining disease susceptibility (Vyse, T.J. and Todd J.A., 1996) although the respective roles of the different MHC Class II⁺ cell types in promoting disease has not yet been clarified. Furthermore, IDDM shows temporal, epidemic-like variations and the clinical disease exhibits preferential seasonal onset (Karvonen et al., 1993). Recently, Conrad et al. (1994) provided evidence for

superantigen involvement in IDDM aetiology and postulated that viruses may be the modifying agent responsible for the presence of superantigen on diabetic islets.

Genetic background also has an important influence in multiple sclerosis. In addition, Perron et al (Perron et al, 1997) have recently identified a retrovirus which can be isolated from cells of multiple sclerosis patients. Whether the retrovirus contributes as a causative agent of multiple sclerosis or as a link in the pathogenic process, or whether it is merely an epiphenomenon, has not been identified. No superantigen activity of the retrovirus has been identified.

It is an aim of the present invention to identify agents implicated in the pathogenesis of human autoimmune diseases, such as IDDM, and on the basis of these agents to provide reliable diagnostic procedures and therapeutic or prophylactic substances and compositions.

These objectives are met by the provision, according to the invention, of diagnostic procedures involving the detection of expressed retroviruses having superantigen (SAg) function, these retroviruses being directly involved in the pathogenesis of human autoimmune disease by activation of autoreactive T-cells. Compounds and compositions capable of blocking SAg function or production are also provided as therapeutic and prophylactic agents in the treatment of autoimmune disease.

The present invention is based on the discovery, by the present inventors that superantigens (SAGs) encoded by retroviruses, particularly endogenous retroviruses, play a major role in the pathogenesis of autoimmune disease, very likely by activating autoreactive T-cells.

Superantigens (SAGs) (Choi et al, 1989 ; White et al, 1989) are microbial proteins able to mediate interactions between MHC Class II⁺ - and polyclonal T-cells resulting in reciprocal activation (Acha-Orbea et al, 1991 ; Choi et al, 1991 ; Fleischer and Schrezenmeier, 1988). Their function is restricted by only two absolute requirements : the presence of MHC Class II on the surface of the presenting cells and the expression of one or more defined Variable (V)- β T cell receptor (TCR) chain(s) on T cells.

The potential role of SAGs in human diseases is ill-defined. Bacterial SAGs have been proposed to be associated with the pathogenesis of autoimmune disease (White et al, 1989). However, although pathogen disease associations have been described, none of these have as yet implicated a pathogen-encoded SAG (Howell et al, 1991 ; Paliard et al, 1991). A SAG-like activity resembling the one encoded by MMTV has been reported to be associated with herpesvirus infections (Dobrescu et al, 1995 ; Sutkowski et al, 1996). However, in none of these two systems has it been demonstrated that the SAG activity is actually encoded by the infectious agent. SAG activity has been reported in patients having Type

I diabetes (Conrad et al 1994). However, the origin of the SAg activity is not identified.

In the framework of the present invention, the inventors have identified the source of SAg activity in IDDM patients as being a novel endogenous retrovirus, (HERV) designated IDDKK_{1,2}-22. This retrovirus is related to, but distinct from mouse mammary tumor virus (MMTV). It is ubiquitous in the human genome but is only expressed in diabetic individuals, possibly in response to a particular environmental stimulus. The HERV encodes superantigen (SAg) activity within the env gene. Expression of the SAg gives rise to preferential expansion of V β -7 T-cell receptor positive T-cells, some of which are very likely to be autoreactive. Thus the expression of self-SAg leads to systemic activation of a sub-set of T-lymphocytes, among which autoreactive T-cells, will in turn give rise to organ-specific autoimmune disease.

The involvement of retroviral SAg, particularly endogenous retroviral SAg in autoimmune disease is unexpected. Indeed, endogenous retroviruses (HERV) form an integral part of the human genome. If expressed from birth, any autoreactive T-cells activated by expression of a retroviral SAg should be deleted as part of the normal development of the immune system (thymic deletion). However, in the case of autoimmune diseases such as diabetes, the expression of the retrovirus, and hence of the encoded SAg, occurs only later in life, leading to the proliferation of autoreactive T-cells.

To identify the microbial agent responsible for SAg activity in diabetes, the present inventors have developed a novel primer-extension technique. This method can be used to isolate and identify, in a sample of polyadenylated RNA, any expressed, previously unidentified retroviral RNA, particularly retroviruses having SAg activity and being involved in human autoimmune disease. This strategy relies on the following three characteristic features of functional retroviruses. First, retroviral genomes contain a primer binding site (PBS) near their 5' end. Cellular tRNAs anneal to the PBS and serve as primers for Reverse Transcriptase (reviewed by Whitcomb and Hughes, 1992). Second, the R (repeat) sequence is repeated at the 5' and 3' ends of the viral RNA (Temin, 1981). Third, the RT-RNase H region of the pol gene is the most conserved sequence among different retroelements (McClure et al., 1988; Xiong and Eickbusch, 1990). The method comprises the following steps :

i) isolation of the 5' R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3' primer being complementary to known « primer binding sites » (pbs).

ii) isolation of the 3' R-poly(A) ends corresponding to the 5' R-U5 ends, by use of primers specific for the R regions isolated in step i).

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region.

iv) amplification of the 5' moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3' end of the central pol region isolated in step iii).

v) amplification of the 3' moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3' R-poly(A) sequences isolated in step ii).

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

Once an expressed retrovirus has been identified, its SAg activity can be tested by contacting a biological sample containing MHC Class II⁺ cells expressing the putative SAg activity, with cells bearing one or more variable (V)- β T-cell receptor (TCR) chains and detecting preferential proliferation of a V β subset.

The techniques developed by the inventors to elucidate SAg involvement in IDDM, can be used to identify the possible involvement of expressed retrovirus and encoded SAg activity in other autoimmune diseases. The characterisation of the retrovirus and its SAg can then be made, and the particular V β -T cell receptor chain activation associated with the SAg can be identified. A given autoimmune disease can thus be defined by reference to a characterised retroviral SAg specifically associated with the disease, and to the

V β -specificity or specificities. In certain autoimmune diseases, such as multiple sclerosis, it is known that T-cells with different V β specificities can be involved in the recognition of the same immunodominant autoantigen, M.B.P. (Wucherpfennig K.W. et al, Science 1990, 25, 1016-1019). Once this « profile » has been determined, specific diagnostic, therapeutic and prophylactic tools can be elaborated for each autoimmune disease involving retroviral SAg-stimulation of autoreactive T-cells.

The present invention involves, in a first embodiment, methods of diagnosis of autoimmune disease based on the specific expression, in autoimmune patients, of retroviruses having SAg activity.

The methods of diagnosis of the present invention are advantageous in so far as they are highly specific, distinguishing between expressed and non-expressed viral nucleic acid, and can thus be reliably used even when the pathological agent is a ubiquitous endogenous retrovirus. They can be carried out on easily accessible biological samples, such as blood or plasma, without extensive pre-treatment. The diagnostic methods of the invention detect disease-specific expression of the retrovirus and can thus be applied before appearance of clinical symptoms, for example on genetically predisposed individuals. This allows suitable therapy to be initiated before autoimmune destruction of a particular target tissue occurs.

In the context of the present invention, the following terms encompass the following meanings :

- a « human autoimmune disease » is defined as a polygenic disease characterised by the selective destruction of defined tissues mediated by the immune system. Epidemiological and genetic evidence also suggests the involvement of environmental factors.
- a « human endogenous retrovirus » (HERV) is a retrovirus which is present in the form of proviral DNA integrated into the genome of all normal cells and is transmitted by Mendelian inheritance patterns. Such proviruses are products of rare infection and integration events of the retrovirus under consideration into germ cells of the ancestors of the host. Most endogenous retroviruses are transcriptionally silent or defective, but may be activated under certain conditions. Expression of the HERV may range from transcription of selected viral genes to production of complete viral particles, which may be infectious or non-infectious. Indeed, variants of HERV viruses may arise which are capable of an exogenous viral replication cycle, although direct experimental evidence for an exogenous life cycle is still missing. Thus, in some cases, endogenous retroviruses may also be present as exogenous retroviruses. These variants are included in the term « HERV » for the purposes of the invention. In the context of the invention, « human endogenous retrovirus » includes proviral DNA corresponding to a full retrovirus as represented schematically in Fig. 2A, comprising two LTR's, gag, pol and env, and further includes remnants or « scars » of such a full

retrovirus which have arisen as a results of deletions in the retroviral DNA. Such remnants include fragments of the structure depicted in Fig. 2A, and have a minimal size of one LTR. Typically, the HERVs have at least one LTR, preferably two, and all or part of gag, pol or env.

- a Superantigen is a substance, normally a protein, of microbial origin that binds to major histocompatibility complex (MHC) Class II molecules and stimulates T-cell, via interaction with the V β domain of the T-cell receptor (TCR). SAGs have the particular characteristic of being able to interact with a large proportion of the T-cell repertoire, i.e. all the members of a given V β subset or « family », or even with more than one V β subset, rather than with single, molecular clones from distinct V β families as is the case with a conventional (MHC-restricted) antigen. The superantigen is said to have a mitogenic effect that is MHC Class II dependent but MHC-unrestricted. SAGs require cells that express MHC Class II for stimulation of T-cells to occur.
- « SAg activity » signifies a capacity to stimulate T-cells in an MHC-dependent but MHC-unrestricted manner. In the context of the invention, SAg activity can be detected in a functional assay by measuring either IL-2 release by activated T-cells, or proliferation of activated T-cells.
- a retrovirus having SAg activity is said to be « associated with » a given autoimmune disease when

expressed retroviral RNA can be found specifically in biological samples of autoimmune patients (ie the expressed retroviral RNA is not found in individuals free of autoimmune disease). Preferably « associated with » further signifies in this context that retroviral SAg activation of a V β subset gives rise directly or indirectly to proliferation of autoreactive T-cells targeting tissue characteristic of the autoimmune disease. Blockage of SAg activity thus normally prevents generation of autoreactive T-cells. Disease « association » with Sag can also be defined immunologically or genetically : immunological association means that a particular disease-associated HLA haplotype is permissive for Sag, whereas resistant haplotypes are permissive for Sag inhibition. Genetic association implies a polymorphism in either the expression pattern of Sag or in the amino acid sequence of Sag, with Sag alleles exhibiting different degree of susceptibility to the disease.

- cells which « functionally express » Sag are cells which express Sag in a manner suitable for giving rise to MHC-dependent, MHC-unrestricted T-cell stimulation in vitro or in vivo. This requires that the cell be MHC II⁺ or that it has been made MHC II⁺ by induction by agents such as IFN- γ .

More particularly, in a first embodiment, the present invention relates to a process for the diagnosis of a human autoimmune disease, including pre-symptomatic diagnosis, said human autoimmune disease

being associated with human retrovirus having Superantigen (SAg) activity, comprising specifically detecting in a biological sample of human origin at least one of the following :

- I : the mRNA of an expressed human retrovirus known to have Superantigen (SAg) activity, or fragments of such expressed retroviral mRNA, said retrovirus being associated with a given autoimmune disease, or
- II : protein expressed by said retrovirus, or
- III : antibodies specific to the proteins expressed by said retrovirus, or
- IV : SAg activity specifically associated with the autoimmune disease.

Thus, the diagnosis of a given autoimmune disease can be made, according to the invention, by one or more of four methods (I to IV), each involving the detection of a specific aspect of the expression of a SAg-encoding retrovirus known to be associated with the autoimmune disease, particularly an endogenous retrovirus. Detection of any of the species (I) to (IV) as listed above is indicative of the presence of the autoimmune disease specifically associated with the endogenous retrovirus under consideration or of imminent onset of the disease.

Each of the four possible methods I to IV of diagnosis of human autoimmune disease will be described in detail below.

According to method I, the autoimmune disease is diagnosed by specifically detecting in a biological

sample the mRNA of an expressed human retrovirus known to have SAg activity.

Specific detection of retroviral expressed mRNA is preferably carried out using nucleic acid amplification with viral specific primers which discriminate between proviral DNA and expressed RNA template. This is of particular importance when the retrovirus associated with the autoimmune disease is an endogenous retrovirus. Indeed in such cases, the proviral DNA is present in all human cells, whether or not the autoimmune disease is present. False positives would be obtained if a detection method were used which does not distinguish between proviral DNA and transcribe mRNA.

The biological sample to be used for specific mRNA detection according to the invention may be any body fluid or tissue but is preferably plasma or blood. Normally, total RNA is extracted from the sample using conventional techniques. DNase treatment may be carried out to reduce contaminating cellular DNA.

By performing the amplification on total RNA samples, the effects of contaminating DNA are reduced but not eliminated, even after treatment by DNase. The method of the present invention allows selective amplification of expressed viral RNA transcripts using at least one m-RNA specific primer, for example a poly-A specific primer, even in the presence of contaminating viral DNA in the sample. The poly-A specific primer is specific for the poly-A signals present in the R-poly(A) sequences and the 3' extremity

of the retrovirus (see for example Figure 2A step 5 and Figure 2C).

It has surprisingly been found that a poly-A-specific primer having from four to 25 T's for example 5 or 20 T's is optimal for the purposes of the present invention.

The mRNA specific amplification requires a reverse transcriptase (RT) step, for which the poly A-specific primer is also be used.

The second primer in the PCR step is generally complementary to the U3 region. When the amplification product has a size of about 300 to 500 nucleotides, the conditions applied for the amplification (PCR) step are normally the following :

- | | | |
|----------------------------|---------|-------------|
| i) reverse transcriptase : | 50°C | 30 minutes |
| | | |
| ii) amplification | : 94°C | 2 minutes |
| (for a total | 94°C | 30 secondes |
| of 10 cycles) | 68°C | 30 secondes |
| | - 1.3°C | each cycle |
| | 68°C | 45 secondes |
| | | |
| iii) amplification | : 94°C | 30 secondes |
| (for a total | 55°C | 30 secondes |
| 25 cycles) | 68°C | 45 secondes |

The amplified material is subjected to gel electrophoresis and hybridised with suitable probes, for example generated from the U3 region.

By performing the mRNA specific detection of the invention, the presence of a given expressed retrovirus can be reliably determined in a biological sample. For endogenous retroviruses expression generally indicates onset of the disease process. This can be detected well before the apparition of any clinical symptoms. The diagnosis of the invention can thus be used to detect onset of the disease process, enabling treatment to be administered before irreversible autoimmune attack occurs.

The invention also encompasses pro-viral specific detection of retroviral DNA, and simultaneous detection of both expressed retroviral m-RNA and proviral DNA. Details of these methods are given in Figure 2D and 2E, and associated legends. Specific proviral DNA detection can be used on healthy biological samples to confirm the endogenous nature of the retrovirus. the assay detecting both retroviral mRNA and proviral DNA can be used as an internal standard.

According to a preferred embodiment of the invention, the autoimmune disease detected is IDDM. The present inventors have identified, a human endogenous retrovirus associated with IDDM. This novel retrovirus (called IDDMK_{1,2}-22) has SAg activity encoded in the NH₂ terminal portion of the env gene, causing preferential proliferation of V β 7 - TCR chain bearing T-cells.

IDDMK_{1,2}-22 comprises the 5' LTR, 3' LTR and env-encoding sequences shown in Figures 7A, 7B and 7C respectively, and further comprises gag-encoding sequences. The SAg portion of the env protein occurs

within the sequences shown in Figure 7D or 7G, particularly 7G.

Diagnosis of IDDM by specific detection of expressed retroviral RNA is carried out using a polyA specific probe of the type :

5' TTTTGAGTCCCCTTAGTATTTATT 3'

or similar sequence specifically hybridising to the polyA region of IDDMK_{1,2}-22 type retroviruses, having at least 90% sequence identity with the IDDMK_{1,2}-22 and having SAg activity.

According to a second embodiment (II) of the invention, the human autoimmune disease associated with a retroviral SAg is diagnosed by specifically detecting protein expressed by the retrovirus, particularly gag, pol or env. In the case of endogenous retroviruses, the expressed proteins may be slightly different from the expected products as a result of read-through phenomena and possibly reading-frame shifts. Preferably, the expressed protein is detected in the biological sample, such as blood or plasma, using antibodies, particularly monoclonal antibodies, specific for the said protein. A Western-like procedure is particularly preferred, but other antibody-based recognition assays may be used.

In the case of IDDM, a preferred diagnostic method comprises the detection of a protein encoded by the env gene, as shown in Figure 7C, 7D or 7G, or the pol protein shown in Figure 7H, or the IDDMK_{1,2}-22 GAG protein. Alternatively, proteins having at least

approximately 90 % homology with these proteins, or proteins arising from read-through of internal stop codons, possibly with frame-shift, particularly a -1 frame shift, occurring immediately after the internal stop codon. Fragments of any of these proteins having at least 6, and preferably at least 10 amino acids, for example 6-20, or 10-15 amino acids, may also be detected. Preferred proteins for this type of diagnostic assay are those having SAg activity. It is also possible to detect retroviral particles when produced.

According to a third embodiment (III) of the invention, the autoimmune disease is diagnosed by detecting in a biological sample, antibodies specific for the protein expressed by the associated retrovirus.

Detection of antibodies specific for these proteins is normally carried out by use of the corresponding retroviral protein or fragments thereof having at least 6 amino-acids, preferably at least 10, for example 6-25 amino acids. The proteins are typically Gag, Pol or Env or fragments thereof and may or may not have superantigen activity. The retroviral proteins used in the detection of the specific antibodies may be recombinant proteins obtained by introducing viral DNA encoding the appropriate part of the retrovirus into eukaryotic cell and the conditions allowing the DNA to be expressed and recovering the said protein.

In the context of the present invention, the terms "antibodies specific for retroviral proteins"

signifies that the antibodies show no significant cross reaction with any other proteins likely to occur in the biological sample. Generally, such antibodies specifically bind to an epitope which occurs exclusively on the retroviral protein in question. The antibodies may recognize the retroviral protein having SAg activity as presented by the M.H.C class II molecule.

Detection of specific antibodies may be carried out using conventional techniques such as sandwich assays, etc. Western blotting or other antibody-based recognition system may be used.

According to the fourth embodiment of the invention, the autoimmune disease is diagnosed by detecting, in a biological sample, SAg activity specifically associated with the autoimmune disease. This is done by carrying out a functional assay in which a biological fluid sample containing MHC class II+ cells, for example Antigen Presenting Cells (APC) such as dendritic cells is contacted with cells bearing one or more variable β -T-receptor chains and detecting preferential proliferation of the $V\beta$ subset characteristic of said autoimmune disease. Typically, this method of diagnosis is combined with one or more of the methods (I), (II), (III) as described earlier to maximise specificity.

The biological sample according to this variant of the invention is typically blood and necessarily contains MHC class II+ cells such as B-lymphocytes, monocytes, macrophages or dendritic cells which have the

capacity to bind the superantigen and enable it to elicit its superantigen activity. MHC class II content of the biological sample may be boosted by addition of agents such as IFN-gamma.

The biological fluid sample is contacted with cells bearing the V β -T receptors belonging to a variety of different families or subsets in order to detect which of the V β subsets is stimulated by the putative SAg, for example V- β 2, 3, 7, 8, 9 13 and 17. Within any one V- β family it is advantageous to use V- β chains having junctional diversity in order to confirm superantigen activity rather than nominal antigen activity.

The cells bearing the V- β receptor chains may be either an unselected population of T-cells or T-cell hybridoma. If unselected T-cells are used, the diagnostic process is normally carried out in the following manner : the biological sample containing MHC Class II+ cells is contacted with the T-cells for approximately 3 days. A growth factor such as Interleukin 2 (IL-2) which selectively amplifies activated T-cells is then added. Enrichment of a particular V- β family or families is measured using monoclonal antibodies against the TCR- β -chain. Only amplified cells are thus detected. The monoclonal antibodies are generally conjugated with a detectable marker such as a fluorochrome. The assay can be made T-cell specific by use of a second antibody, anti CD3, specifically recognizing the CD3-receptor.

T-cell hybridoma bearing defined T-cell receptor may also be used in the functional or cell-based assay for SAg activity. An example of commercially available cells of this type are given in *B. Fleischer et al. Infect. Immun.* 64, 987-994, 1996. Such cell-lines are available from Immunotech, Marseille, France. According to this variant, activation of a particular family of V- β hybridoma leads to release of IL-2. IL2 release is therefore measured as read-out using conventional techniques. A specific example of this procedure for diabetes is illustrated in Figure 9. The basic methodology is adapted for other autoimmune diseases by employing T-cell receptor cells of the appropriate type for that disease.

For diabetes, detection of SAg activity will normally lead to preferential proliferation of the V- β 7 subset. For other autoimmune diseases, other V- β subsets may be proliferated.

According to another aspect of the present invention, there is provided human endogenous retroviruses having superantigen activity and being associated with human auto immune disease. Such retroviruses which may be of the HERV-K family, or otherwise, are obtainable from RNA prepared from a biological sample of human origin, by carrying out the following steps :

- i) isolation of the 5' R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3' primer being complementary to known « primer binding sites » (pbs) ;

ii) isolation of the 3' R-poly(A) ends corresponding to the 5' R-U5 ends, by use of primers specific for the R regions isolated in step i) ;

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region ;

iv) amplification of the 5' moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3' end of the central pol region isolated in step iii) ;

v) amplification of the 3' moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3' R-poly(A) sequences isolated in step ii) ;

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

A preferred human endogenous retrovirus of the invention is IDDMK 1,2 22 comprising each of the sequences illustrated in figures 7A, 7B, 7C or sequences having at least 90 % identity with these sequences, and further comprising GAG-encoding sequences, and sequences encoding POL as shown in figure 7H. This retrovirus has a size of approximately of 8.5 kb, has SAg activity encoded within the Env region as shown in figure 7C and 7E and gives rise to V- β 7 specific proliferation.

The invention also relates to proviral DNA of a retrovirus having superantigen activity and being associated with an autoimmune disease. Such proviral DNA is naturally found integrated into the human genome. The proviral DNA may be obtained from a biological sample of human origin by :

- i) obtaining retroviral RNA according to the method of claim 13, and further,
- ii) generating a series of DNA probes from the retroviral RNA obtained in i);
- iii) hybridising under stringent conditions, the probes on a genomic human DNA library ;
- iv) isolation of the genomic sequences hybridising with the probes.

The invention also relates to nucleic acid molecules (RNA, DNA or cDNA) comprising fragments of the retroviral RNA or DNA described above, having at least 20 nucleotides and preferably at least 40. The fragments may be specific for a given retrovirus, specific signifying a homology of less than 20 % with other human or non-human retroviruses.

Preferred nucleic acid molecules of the invention encode SAg activity particularly SAg activity, responsible for the proliferation of autoreactive T-cells. If the region of the viral genome encoding the SAg activity is unknown, the particular region may be identified by :

- i) transfecting expressed retroviral DNA or portions thereof into MHC Class II⁺ antigen presenting

cells under conditions in which the viral DNA is expressed,

ii) contacting the MHC class II⁺ transfectants with cells bearing one or more defined (V)- β T-cell receptor chains, and

iii) determining whether the transfectant is capable of inducing preferential proliferation of a V β subset, the capacity to induce preferential proliferation being indicative of SAg activity within the transfected DNA or portion thereof. Proliferation may be measured by determination of 3H-thymidine incorporation (see Examples methods and materials).

The nucleic acid molecule encoding SAg activity may be derived from an endogenous human retrovirus. It typically corresponds to an open reading frame of the retrovirus and may contain at least one internal stop codon or may be a synthetic mutant in which 1 or 2 nucleotides have been added or deleted to remove the stop codon and modify the reading frame.

Preferably, the nucleic acid of the invention comprises or consists of all or part of the env gene (encoding the envelope glycoprotein) of an endogenous human retrovirus associated with autoimmune disease. The env - encoded protein is particularly likely to have SAg activity, as exemplified by the IDDM HERV. Synthetic or recombinant nucleic acids corresponding to the env genes or fragments thereof are also within the scope of the invention.

The nucleic acid molecules of the invention may comprise ribozymes or antisense molecules to the retrovirus involved in autoimmune disease.

The invention also relates to nucleic acid molecules capable of hybridizing in stringent conditions with retroviral DNA or RNA. Typical stringent conditions are those where the combination of temperature and salt concentration chosen to be approximately 12-20°C below the T_m (melting temperature) of the hybrid under study.

Such nucleic acid molecules may be labelled with conventional labelling means to act as probes or, alternatively, may be used as primers in nucleic acid amplification reactions.

Preferred nucleic acid molecules of the invention are illustrated in figures 7A, 7B, 7C, 7D, 7E, 7G and also encompass nucleic acid sequences encoding the POL protein shown in figure 7H, and the GAG protein. Sequences exhibiting at least 90 % homology with any of the afore-mentioned sequences are also comprised within the invention or fragments of any of these sequences having at least 20 and preferably at least 30 nucleotides.

The Env encoding sequence shown in figure 7C is particularly preferred, as well as the nucleic acid encoding the Env/F-S SAg protein shown in figures 7G and 7E. A preferred nucleic acid molecule is a molecule encoding the Env/F-S SAg protein wherein the first internal stop codon (shown underlined in figure 7C), is mutated by insertion of an extra T (at position 517 in

Figure 7G underlined) to eliminate premature translational stop, the resulting sequence being then in the correct reading frame to encode the COOH terminal extension (shown underlined in Figure 7G). This protein arises naturally from read-through together with a -1 frame shift, but this process is inefficient. The synthetic T'-inserted cDNA provides an efficient way of producing the SAg molecule shown in Figure 7G. The single reading frame in this « synthetic » molecule thus corresponds to two different reading frames separated by a stop codon in the natural molecule. Nucleic acid molecules encoding an HERV env and including minus 1, plus 1 frameshifts and termination suppression (0 frame) are thus particularly preferred embodiments of the invention.

The invention further relates to proteins expressed by human endogenous retroviruses having SAg activity and being associated with human autoimmune disease. Peptides or fragments of these proteins having at least 6 and preferably at least 10 aminoacids, for example 6-50 or 10-30 amino acids, are also included within the scope of the invention. Such proteins may be Gag, Pol or Env proteins or may be encoded by any Open Reading Frame situated elsewhere in the viral genome. These proteins may or may not present SAg activity. Particularly preferred proteins of the invention have SAg activity. Examples of SAg proteins of the invention are proteins encoded by the env gene of HERV, for example that shown in Figure 7G.

The proteins having SAg activity may naturally result from a premature translational stop and possibly also from a translational frameshift. Endogenous retroviral ORFs typically contain a number of internal stop codons, which often render the HERV defective. It has been discovered by the present inventors that, in some cases, retroviral expression products having SAg activity result from read-through transcription of the ORF, possibly also accompanied by a reading frame shift. Consequently, the proteins exhibiting SAg activity are not, in these cases, the expected expression products of the retrovirus.

It may therefore be deduced that open reading frames of retroviruses associated with human autoimmune disease which contain at least one internal translational stop codon are among potential candidates for SAg activity. The proteins produced by premature translational stop may have an additional carboxy-terminal extension resulting from translational frame shift, for example -1 or -2 or +1 or +2 translational frame shift. Such a protein is illustrated in figure 7G. Further preferred proteins of the invention are the proteins encoded by synthetic cDNA, corresponding to the in-frame fusion of two normally different reading frames, together with mutation of the internal stop codon. These artificial open-reading frames are made by inserting or deleting one or two nucleotides in the coding sequence at the site where frame-shift occurs naturally, thus « correcting » the reading frame and

enabling efficient production of a protein which is naturally only produced very inefficiently.

Other proteins of the invention are those comprising the aminoacid sequences shown in figure 7D, 7F, 7H or an aminoacid sequence having at least 80 % and preferably at least 90 % homology with the illustrated sequences or fragments of these sequences having at least 6 and preferably at least 10 aminoacids. The proteins of the invention may be made by synthetic or recombinant techniques.

The invention also relates to antibodies capable of specifically recognizing a protein according to the invention. These antibodies are preferably monoclonal. Preferred antibodies are those which specifically recognize a retroviral protein having SAg activity and which have the capacity to block SAg activity. The capacity of the antibody to block SAg activity may be tested by introducing the antibody under test into an assay system comprising :

- i) MHC Class II⁺ cells expressing retroviral protein having SAg activity and
- ii) cells bearing V β -T cell receptor chains of the family or families specifically stimulated by the HERV SAg expressed by the MHC Class II⁺ cells, and determining the capacity of the substance under test to diminish or block V β -specific stimulation by the HERV SAg.

The steps described below involve the use of SAg-expressing transfectant cells such as those described

in the examples, to inhibit the effect of Sag in vitro and in vivo. The example applies to the Sag expressed by the IDDM-associated HERV, as well as to other Sags, encoded by HERV associated with other autoimmune diseases, such as multiple sclerosis, and previously identified as Sag by a functional T cell activation assay as described earlier.

Mabs directed against the Sag protein (or portion of it) are generated by standard procedures used to generate antibodies against cell surface antigens. Mice are immunised with mouse cells expressing both Sag and MHC class II (such as a Sag-transfected mouse B cell line described in the examples below). After fusion with hybridoma cell lines, supernatants are screened for the presence of anti-Sag antibodies on microtiter plates for reactivity to Sag transfectants cells, with non-transfected cells as negative controls. Only Mabs with reactivity specific for Sag expressing cells are selected.

All such Mabs, either as culture supernatants or as ascites fluid, are then tested for their ability to block the Sag activity, as assayed by the T cell assay in the presence of Sag-expressing human MHC class II positive transfectants, as described in Example 4 below. A preferred version of this assay makes use of V β -specific hybridomas as T cell targets for read out. Controls are blocking of the same assay by anti-HLA-DR Mabs, which is known to inhibit the Sag effect on T cell activation. Mabs capable of efficiently blocking

the V β -specific Sag effect, when tested at several dilutions, are selected as anti-Sag blocking Mabs.

As well as monoclonal antibodies capable of inhibiting IDDM Sag, this generation and selection of anti-Sag blocking Mabs can be achieved in the case of any HERV-encoded Sag associated with other autoimmune diseases, once such a HERV-encoded Sag has been demonstrated.

Sufficient numbers of anti-Sag Mabs are screened in the functional assay to identify anti-Sag Mabs with optimal Sag blocking activity, in terms of T cell activation (see for example Figure 9). Selected Sag blocking Mabs are then converted into their « humanised » counterpart by standard CDR grafting methodology (a procedure performed for a fee under contract by numerous companies). A humanised anti-Sag blocking Mab, directed against the IDDM associated Sag or against any Sag encoded by another HERV associated with autoimmunity, can then be tested clinically in patients. In the case of IDDM, early diagnosed patients are selected and protection against progressive requirement for insulin therapy is followed as an index of efficacy. In the case of other autoimmune diseases, efficacy of the anti-Sag Mab is followed with reference to the relevant clinical parameters.

The invention also relates to cells transfected with and expressing human endogenous retrovirus having SAg activity and being associated with a human autoimmune disease. The cells may be preferably human cells other than the naturally occurring cells from

auto-immune patients and may also include other type of eukaryotic cells such as monkey, mouse or other higher eukaryotes. The cells may be established cell-lines and are preferably MHC class II⁺, or MHC II⁺-inducible, such as β -lymphocytes and monocytes. Non-human higher eukaryotic cell-lines (e.g. mouse) stably transfected with the HERV Sags of the invention (as exemplified in Example 6 below) have been found to specifically stimulate in vitro human v β -T cells of the specificity normally associated with the HERV Sag in vivo. The stimulation is coreceptor independent (CD4 and CD8). This specific T-cell stimulation can also be observed in vivo upon injection of the transfectants into non-human animals. A transgenic animal model for the human autoimmune disease is therefore technically feasible. The transgenic animal is made according to conventional techniques and includes in its genome, nucleic acid encoding the HERV Sags of the invention.

A further important aspect of the invention relates to the identification of substances capable of blocking or inhibiting SAg activity. These substances are used in prophylactic and therapeutic treatment of autoimmune diseases involving retroviral SAg activity. The invention thus concerns methods for treating or preventing autoimmune disease, for example IDDM, by administering effective amounts of substances capable of blocking Sag activity associated with expression of a human endogenous retrovirus. The substances may be antibodies, proteins, peptides, derivatives of the HERV, derivatives of the Sag or small chemical

molecules. The invention also relates to pharmaceutical compositions comprising these substances in association with physiological acceptable carriers, and to methods for the preparation of medicaments for use in therapy or prevention of autoimmune disease using these substances.

Further, this aspect of the invention includes a process for identifying substances capable of blocking or inhibiting SAg activity of an endogenous retrovirus associated with autoimmune disease, comprising introducing the substance under test into an assay system comprising :

- i) MHC Class II⁺ cells functionally expressing retroviral protein having SAg activity and ;
- ii) cells bearing V β -T cell receptor chains of the family or families specifically stimulated by the HERV SAg expressed by the MHC Class II⁺ cells, and determining the capacity of the substance under test to diminish or block V β -specific stimulation by the HERV SAg,

The cells bearing the β -T cell receptors and the MHC Class II⁺ cells may be those described earlier. Read-out is IL-2 release.

The substances tested for inhibition or blockage of SAg activity in such screening procedures may be proteins, peptides, antibodies, small molecules, synthetic or naturally occurring, derivatives of the retroviruses themselves, etc... Small molecules may be tested in large amounts using combinatorial chemistry libraries.

The screening procedure may include an additional preliminary step for selecting substances capable of binding to retroviral protein having SAg activity. This additional screening step comprises contacting the substances under test, optionally labelled with detectable marker with the retroviral protein having SAg activity and detecting binding.

The Sags of the invention or a portion thereof may be used for the identification of low molecular weight inhibitor molecules as drug candidates.

The rationale is that because HERV encoded Sags are the product of ancient infectious agents, they are not indispensable to humans and can thus be inhibited without adverse side effects.

Inhibitors of Sag, as potential drug candidates, are preferably identified by a two step process :

In the first step, compatible with large scale, high throughput, screening of collections (« libraries ») of small molecular weight molecules, the recombinant Sag protein (or portion of it) is used in a screening assay for molecules capable of simply binding to the Sag protein (« ligands »). Such high throughput screening assays are routinely performed by companies such as Novalon Inc or Scriptgen Inc, and are based either on competition for binding of peptides to the target protein or on changes in protein conformation induced by binding of a ligand to the target protein. Such primary high throughput screening for high affinity ligands capable of binding to a

target recombinant protein are available commercially, under contract, from such companies as Novalon or Scriptgen. This screening method requires that a HERV protein with Sag activity, and knowledge of such an activity, be available.

In the second step, any low molecular weight molecule identified as described above as capable of binding to the Sag protein, is tested in the functional Sag assay consisting of human MHC class II positive Sag transfectants and responding V β -specific T cells (preferably hybridomas), as described herein. Positive control for Sag inhibition is an anti-HLA-DR Mab, known to inhibit the Sag effect. All candidate molecules are thus tested, at different concentrations, for a quantitative assessment their anti-Sag inhibitory efficacy.

This example can apply to the Sag encoded by the IDDM-associated HERV described herein, as well as to any other Sag discovered to be encoded by another HERV associated with another autoimmune disease.

This screening procedure relies upon the availability of a Sag and of a Sag functional assay according to the invention, but it otherwise relies on commercially available steps. Compounds exhibiting anti-Sag inhibitory effects are then tested for obvious toxicity and pharmacokinetics assays, in order to determine if they represent valuable drug candidates.

Once a substance or a composition of substances has been identified which is capable of blocking or inhibiting SAg activity, its mode of action may be

identified particularly its capacity to block transcription or translation of SAg encoding sequences. This capacity can be tested by carrying out a process comprising the following steps :

- i) contacting the substance under test with cells expressing retroviral protein having SAg activity, as previously defined, and
- ii) detecting loss of SAg protein expression using SAg protein markers such as specific, labelled anti-SAg antibodies.

The antibodies used in such a detection process are of the type described earlier.

The invention also relates to a kit for screening substances capable of blocking SAg activity of an endogenous retrovirus associated with an autoimmune disease, or of blocking transcription or translation of the retroviral SAg protein. The kit comprises :

- MHC Class II⁺ cells transformed with and expressing retroviral SAg according to the invention ;
- cells bearing V β T-cell receptor chains of the family or families specifically stimulated by the HERV SAg ;
- means to detect specific V β stimulation by HERV SAg ;
- optionally, labelled antibodies specifically binding to the retroviral SAg.

According to a further important aspect of the invention, there is provided a protein or peptide derived from an autoimmune related retroviral SAg as previously defined wherein the protein is modified so

as to be essentially devoid of SAg activity, thereby no longer being capable of significantly activating auto-reactive T-cells. Such modified proteins are however capable of generating an immune response against SAg, the immune response involving either antibodies and/or T-cells responses. The immunogenic properties of the modified proteins are thus conserved with respect with the authentic SAg.

Such modified immunogenic proteins may be obtained by a number of conventional treatments of the SAg protein, for example by denaturation, by truncation or by mutation involving deletion, insertion or replacement of aminoacids. Modified SAg proteins being essentially devoid of SAg activity but capable of generating an immune response against SAg include the truncations of the SAg protein, either at the amino or carboxyterminal, and may involve truncations of about 5-30 aminoacids at either terminal. A preferred example with respect to the IDDMK 1.2-22 SAg encoded by the Env gene illustrated in Figure 7, particularly in figure 7E and figure 7G, are amino and carboxy terminal truncations of the protein shown in figure 7G, for example truncations of 5, 10, 15, 20, 25 or 30 amino acids. An example of a C-terminal truncation of the IDDMK 1.2-22 SAg protein is the protein shown in figure 7D, involving a truncation of 28 amino acids. The modified protein may be obtained by recombinant or synthetic techniques, or by modifying naturally occurring SAg proteins, for example by physical or chemical treatment.

These proteins are used in the framework of the invention as vaccines, both prophylactic and therapeutic, against autoimmune disease associated with retroviral SAg. The vaccines of the invention comprise an immunogenically effective amount of the immunogenic protein in association with a pharmaceutically acceptable carrier and optionally an adjuvant. The use of these vaccine compositions is particularly advantageous in association with the early diagnosis of the autoimmune disease using the method of the invention. The invention also includes the use of the immunogenic proteins in the preparation of a medicament for prophylactic or therapeutic vaccination against autoimmune diseases.

The rationale behind this prospective immunisation technique is that because HERV encoded Sags are the product of ancient infectious agents, they are not indispensable to humans and can thus be inhibited without adverse side effects.

Identification of suitable anti-sag vaccine proteins or peptides can be made in the following way. Modified forms of the original active Sag protein, including truncated or mutated forms, or even specific peptides derived from the Sag protein, are first tested in the functional Sag assays described above to confirm that they have lost all Sag activity (in terms of T cell activation). These modified forms of Sag are then used to immunise mice (or humans) by standard procedures and with appropriate adjuvants. Extent and efficacy of immunisation is measured, including

circulating anti-Sag antibodies. In a preferred example, eliciting a B cell immune response, by selecting B cell epitopes from the Sag protein as immunogen, is deliberately aimed at.

Successfully immunised animals are then tested for the effect of Sag in vivo by a standard assay, namely the injection of MHC class II positive Sag transfectants (such as the transfectants described in the examples below), known to induce in vivo a V β -specific T cell activation. Successful immunisation against a Sag protein is expected to result in a reduction or in a block of the in vivo Sag-induced T cell activation and proliferation in effectively immunised individuals. This procedure is referred to as anti-Sag vaccination. Immunisation against Sag can be performed in humans, for diabetes, preferably initially in the case of early diagnosed IDDM patients. Efficacy of this novel « vaccination » procedure is monitored by clinical outcome and by reduction of the expected requirements for insulin therapy. In the case of other Sags, encoded by HERV associated with autoimmune diseases other than diabetes, the clinical outcome is monitored accordingly.

The vaccines of the invention can be prepared as injectables, e.g. liquid solutions or suspensions. Solid forms for solution in, or suspension in, a liquid prior to injection also can be prepared. Optionally, the preparation also can be emulsified. The active antigenic ingredient or ingredients can be mixed with excipients which are pharmaceutically acceptable and

compatible with the active ingredient. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants such as aluminium hydroxide or muramyl dipeptide or variations thereof. In the case of peptides, coupling to larger molecules (e.g. KLH or tetanus toxoid) sometimes enhances immunogenicity. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration includes suppositories and, in some cases, oral formulations.

The vaccines of the invention also include nucleic acid vaccines comprising nucleic acid molecules encoding the human retroviral Sag or modified forms of the SAg known to be immunogenic but no longer active as SAGs. The nucleic acid vaccines, particularly DNA vaccines, are usually administered in association with a pharmaceutically acceptable carrier as an intramuscular injection.

The invention also relates to use of substances inhibiting either the retroviral function or the SAg function of the associated retroviruses, or Sag synthesis, in therapy for autoimmune diseases. These substances may be identified by the screening procedures described herein.

The invention further relates to methods for treatment or prevention of autoimmune diseases comprising administering an effective amount of a substance capable of inhibiting retroviral function or a substance capable of inhibiting SAg activity or synthesis.

An examples of compounds inhibiting retroviral function is AZT. Examples of compounds or substances capable of inhibiting SAg activity are antibodies to Sag, or ribozymes or antisense molecules to the SAg-encoding nucleic acid, or small molecules identified by virtue of their ability to inhibit SAg.

The invention also relates to a an exploratory process for detecting human autoimmune disease associated with expression of unidentified human retrovirus Superantigen (SAg), said process comprising at least one of the following steps :

- i) detecting the presence of any expressed retrovirus in a biological sample of human origin ;
- ii) detecting the presence of SAg activity in a biological sample of human origin containing MHC Class II⁺ cells.

This process can be used as a preliminary indication of the involvement of retroviral superantigens in autoimmune disease.

Different aspects of the invention are illustrated in the figures.

Figure 1. Leukocytes from IDDM-patients release Reverse Transcriptase (RT) activity.

(A) Supernatants derived from cultured islets isolated from two patients (Conrad et al., 1994) were assayed for RT-activity, using a half-logarithmic dilution series of purified murine leukemia virus (MLV) RT as a standard (Pyra et al., 1994). Results are expressed as mean \pm 1 SD. Islets and spleen cells from non-diabetic organ donors were cultured either alone, in the presence or absence of mitogen (-/+), or together in mixed allogeneic cultures (time as days in culture prior to collection of the supernatant is indicated below the bars).

(B) Islets and spleen cells from three non diabetic organ donors, from the two patients with acute-onset IDDM, and two patients with chronic IDDM (Conrad et al., 1994) were cultured for 1 week and supernatants were analyzed for the presence of RT-activity. Results are expressed as mean \pm 1 SD for at least three individual measurements.

Figure 2A. Isolation of a single full length retroviral genome, IDDMK_{1,222}, with a six step procedure.

1) cPBS primers (Lys_{1,2}, Lys₃, Pro, Trp) were used to perform a 5' RACE 2) the eight 5' R-U5 sequences obtained in 1) were used to perform a 3' RACE with primers annealing in the R 3) the conserved RT-RNase H region was amplified with degenerate primers 4) the 5' moiety (the predicted size for full length HERV-K-retroviruses is 3.6 kb was amplified by PCR using primers specific for the eight 5' R-U5 sequences in

conjunction with a primer specific for the 3' of the central *pol* region obtained in step 3. The primer specific for the K_{1,222} 5' consistently yielded a fragment of this size, 5) the 3' (the predicted size for HERV-K-retroviruses is 5 kb) was amplified by PCR using a primer specific for the 5' of the central *pol* region isolated in step 3 and primers specific for the poly(A) signals present in the 3' R-poly(A) sequences obtained in step 2. The PCR reaction using a primer specific for the 3' clone K_{1,222} (amplified in step 4) consistently yielded a fragment potentially representing an intact 3' HERV-K moiety of 5 kb, 6) the presence of an intact 8.6 kb retroviral genome containing the overlapping 5' and 3' moieties isolated in steps 4 and 5 was confirmed by PCR using primers specific for its predicted U5 and U3 regions.

Figure 2B. Consensus features of retroviral 5' end sequences (termed STRs). These consensus features are valid for retroviruses with a polyadenylation signal in the R (repeat) region. The R region is characterized by the **AATAAA** or **ATTAAA** polyadenylation signal (bold) followed by 13 to 20 nucleotides and the dinucleotide **CA** or **GA** (bold) at the 3' end of the R region. The beginning of U5 region is defined by a GT- or T-rich sequence (underlined). The 3' end of the U5 region is in all known retroviruses defined by the dinucleotide **CA**, followed by one, two or three nucleotides and the

primer-binding site (PB) - (N) stands for nucleotide, the suffixes x, y, and z for an undefined number.

Figure 2C. Schematic representation of mRNA-specific PCR of IDDMK_{1,2}-22 using a poly (A)-specific probe (Rc-T₍₄₎). Details of this technique are given in the « Experimental Procedure » Section of the Examples. This procedure results in a Reverse-Transcriptase-dependent amplification of retroviral genomes. The products generated can be diminished below background by RNase treatment.

Figure 2D. Schematic representation of IDDMK_{1,2}-22 Provirus-specific PCR. The procedure specifically amplifies proviral 5' and 3' LTRs (long terminal repeats).

The primers used in an RT- control are substituted with either U5-primers 1) 5'ATC CAA CAA CCA Tga Tgg Ag 3' or 2) 5' TCT Cgt Aag gTg CAA Atg Aag 3' at 0.3 µM final concentration in conjunction with the U3-primers using either 3) gTA Aag gAT CAA gTg Ctg TgC 3' or 4) 5' CTT TAC AAA gCA gTA Ttg Ctg C 3' at 0.3 µM final concentration. 0.75 µl of Taq- Pwo- polymerase mix (Boehriner Mannheim, ExpandTM High Fidelity PCR System) are used with a thermocycler profile corresponding to the one described for mRNA-specific RT-PCR and omitting the RT step.

Hybridization is performed with the probe and the methods corresponding those used for mRNA-specific RT-PCR.

Sequence identity is confirmed by sequencing according to standard procedures.

Figure 2E. IDDMK_{1,2}-22 RNA- and Provirus-specific PCR. This procedure will result in amplification products independently of the presence or absence of RT-reactions and reflects the total retroviral RNA- and DNA- templates present in a given sample.

The same conditions as in the proviral specific PCR are used with U3 primers 1) 5' AAC ACT gCg AAA ggC CgC Agg 3' or 2) 5' Agg TAT TgT CCA Agg TTT CTC C 3' in conjunction with R (repeat) primers 3) 5' CTT TAC AAA gCA gTA TTg Ctg C 3' or 4) 5' gTA Aag gAT CAA gTg Ctg TgC 3'. Cycling conditions and primer concentrations are identical to those described for proviral specific PCR.

Figure 2F. IDDMK_{1,222} is an endogenous retrovirus found in the plasma of IDDM patients at disease onset but not in the plasma of healthy controls.

PCR primers pairs were designed that are either specific for the U3-R- or for the U3-R-poly(A)-region of IDDMK_{1,222} (see Experimental Procedures). The U3-R primer pair amplified both viral RNA and DNA, whereas the U3-R-poly(A) primer pair amplified selectively

viral RNA. The amplified material was hybridized with probes generated with the molecularly cloned U3-R region of IDDMK_{1,22}. Signals in the first and third rows correspond to amplification of contaminating DNA present in the plasma of IDDM patients (left hand columns, 1-10) and controls (right hand columns, 1-10) and were as expected RT-independent. In contrast, signals in the second row resulted from the amplification of viral RNA present only in IDDM patients (left hand columns, 1-10) but not in the non diabetic controls (right hand columns, 1-10). This was supported by the absence of amplification products in reactions lacking RT (fourth row, right and left hand columns, 1-10). In addition the signal could be diminished below background by RNase treatment (data not shown). In the fifth row the genomic DNA from IDDM patients and controls was amplified with the U3-R-specific primers. The primer pair specific for the U3-R-poly(A), in turn, did not result in amplification of genomic DNA (data not shown).

Figure 3. Phylogenetic trees of coding and non-coding regions place IDDMK_{1,22} in the HERV-K10 family of HERVs.

(A) IDDMK_{1,22} SU-ENV is most closely related to HERV-K10, and is also related to the B-type retroviruses MMTV and JSRV.

(B) The phylogenetic analysis of the RT region shows that IDDMK_{1,22} belongs to the HERV-K10 family and is

more closely related to B-type retroviruses such as MMTV than to D-type retroviruses such as Simian Mason Pfizer (SMP) or Spumaviridae (SFV). Abbreviations used: SRV-2, Simian retrovirus; JSRV, Jaagsiekte Sheep retrovirus; SFV; Simian foamy virus).

(C) The non-coding LTR region was used to construct a phylogenetic tree of the HERV-K family. $K_{1,21}$ and $K_{1,24}$ (see above) were isolated only as subgenomic or truncated transcripts. $K_{1,21}$ is related to KC4, while $K_{1,24}$ and IDDMK $_{1,22}$ are related to the K10/K18 subfamily. Within this family, $K_{1,24}$ is closely related to K10, whereas IDDMK $_{1,22}$ appears to be more distant.

Figure 4. The pol-env-U3-R region of IDDMK $_{1,22}$ exerts an MHC class II dependent but not MHC restricted mitogenic effect upon transfection in monocytes.

(A). IDDMK $_{1,22}$ is expected to generate two singly spliced subgenomic RNAs, one encoding ENV, and one comprising the U3-R region. The episomal expression vector was engineered to carry a proximal SD downstream of the promoter (pPOL-ENV-U3). Thus, the two naturally expected subgenomic RNAs can also be generated.

(B) Monocytic cell lines do not express MHC class II surface proteins in the absence of induction by Interferon- γ (INF- γ), (reviewed by Mach et al., 1996). The monocyte cell line THP1 was transiently transfected with pPOL-ENV-U3 or with the expression vector alone (pVECTOR). Mitomycin C treated transfectants, either induced with INF- γ for 48 h or non-induced (+/- INF- γ ,

indicated below the x-axis) were cultured with MHC-compatible T cells at different responder : stimulator ratios as indicated below the graphs (T : APC). ^3H -Thymidine incorporation was measured during the last 18 h of a 72 h culture and is given on the y-axis as $n \times 10^3$ cpm. Results are presented as mean \pm 1 SD.

(C) The MHC class II transactivator CIITA mediates INF- γ inducible MHC class II expression (reviewed by Mach et al., 1996). An integrative and stable THP1-CIITA transfectant (THP1-CIITA) was transfected with pVECTOR or pPOL-ENV-UR and was used in functional assays identical to those described in Figure 4B.

(D) Peripheral blood lymphocytes (PBL) from healthy, MHC-unrelated donors (donors I, II and III indicated below the x-axis) were cultured with retroviral (pPOL-ENV-U3) and control transfectants (pVECTOR) at T : non - T ratios as indicated below the graphs (T : APC).

Figure 5. IDDMK_{1,22} mediates a Vb 7-specific SAG-effect.

10^6 T cells/ml were cultured for 3 days with Mitomycin-treated pPOL-ENV-U3 and pVECTOR transfectants at T : non - T ratios as indicated. Twenty U/ml of recombinant IL-2 were then added to the cultures and FACS analysis performed after 3 to 4 days of expansion (Conrad et al., 1994).

(A) THP1 cells were transfected with pPOL-ENV-U3, the stimulated and expanded T cells were stained with anti-

CD3 monoclonal antibodies and an isotype control after 7 days of coculture.

(B) T cells stimulated by THP1 transfected with the vector (pVECTOR) alone were stained with anti-CD3 monoclonal antibodies and the anti Vb 7-specific antibody 3G5.

(C) THP1 cells were transfected with pPOL-ENV-U3, the stimulated T cells were stained with anti-CD3 monoclonal antibodies and the anti Vb 7-antibody 3G5.

Table1. IDDMK_{1,22} mediates a Vb 7-specific SAG-effect. The B lymphoblastoid cell line Raji was stably transfected with either pPOL-ENV-U3 or pVECTOR, and used in functional assays (equivalent to Figure 5) 2 weeks after selection. The monocytic cell line THP1 was cultured for 48 hours after transfection with the same constructs. The percentages of double positive (CD3 and Vb-7, Vb-8, -12) T cells are indicated that were obtained after 1 week of coculture with the respective transfectants (pPOL-ENV-U3 or pVECTOR).

Figure 6. The N-terminal env moiety of IDDMK_{1,22} mediates the SAG-effect.

(A). Based on the construct pPOL-ENV-U3 different deletional mutants were generated that comprised 1) pPOL: the pol gene; 2) pPOL-ENV/TR: the pol - and the N-terminal moiety of the env-gene; 3) pCI-ENV/TR: the N-terminal moiety of env-gene alone.

(B). PBL from MHC unrelated donors were cocultured with Mitomycin C treated THP1 cells as described in Figure 4. The individual transfectants are indicated with the names of the constructs above the bars. (1) pVECTOR, 2) pPOL, 3) pPOL-ENV-U3, 4) pPOL-ENV/TR, 5) pCI-neo, 6) pCI-ENV/TR). One of at least three independent ^3H -Thymidine incorporation experiments with allogeneic T cells stimulated by the individual transfectants is shown. The ratio between T cells and transfectants is indicated below the bars (T : APC).

Figure 7A. IDDMK_{1,22} - 5' LTR.

This figure shows the sequence of the 5' LTR (U3 RU5) of the IDDMK_{1,22} - provirus.

Figure 7B. IDDMK_{1,22} - 3' LTR.

This figure shows the sequence of the 3' LTR (U3 RU5) of the IDDMK_{1,22} provirus.

Figure 7C. IDDMK_{1,22} - env.

This figure shows the full nucleotide sequence of the env coding region, starting with the ATG initiation codon at position 59 (as shown in Figure 7D).

The first internal stop codon TAG at position 518 is underlined corresponding to the codon where, following a -1 frame shift, translation stops to give rise to the protein illustrated in Figure 7D.

The second internal stop codon TAG at position 601 (in frame with the earlier TAG) is also underlined. Translational stop at this codon gives rise to the IDDMK_{1,22} - ENV / FS (SAG) protein illustrated in Figure 7G. The nucleic acid coding for the IDDMK_{1,22} - env/fs (SAG) protein is also shown in Figure 7E.

Figure 7D. The nucleotide and deduced amino acid sequence of IDDMK_{1,22}-SAG.

The minimal stimulatory sequence corresponding to the insert of pCI-ENV/TR comprises a C-terminally truncated protein of 153 amino acids. There is only one ORF with a stop codon at position 518. The first potential start codon in a favorable context is at position 59. Two potential N-linked glycosylation sites are present at positions 106, and 182 respectively. The degree of homology with other retroviral ENV proteins is shown in Figure 3A. No significant homology was detected with the SAG of MMTV or with autoantigens known to be important in IDDM.

Figure 7E. IDDMK_{1,22} - env/fs - sag.

Wild-type Nucleotide sequence coding for the 181 amino acid IDDMK_{1,22} - ENV/FS - SAG protein shown in Figure 7G. To give rise to the SAG protein shown in figure 7G, translation of this nucleotide sequence involves a read-through of the first stop codon at position 518 followed immediately by a -1 frame shift.

Figure 7F. IDDMK_{1,2}22 - ENV.

Deduced amino acid sequence encoded by the full env coding region (as shown in Figure 7B), without frame shift.

The underlined « Z » is the stop site for the 153 amino acid protein shown in Figure 7D.

Figure 7G. Recombinant IDDMK_{1,2}22 ENV/FS (SAG).

With respect to wild-type IDDMK_{1,2}22 env an insertion of a T at position 517 (underlined) results in a predicted protein corresponding to the one expected to be generated by IDDMK_{1,2}22 ENV/FS. The additional predicted C terminal amino acids that characterize ENV-FS are underlined. This protein has marked SAg activity.

Figure 7H. IDDMK_{1,2}22 POL.

Deduced amino acid sequence of the POL protein of IDDMK_{1,2}22.

Figures 8A to 8G illustrate candidate 5' STRs isolated in the first step of the six-step procedure (illustrated in Figure 2A) to isolate putative retroviral genomes from IDDM patients.

Figure 9. Functional assay for the presence of V β 7-IDDM-SAG in PBL.

PBL (peripheral blood lymphocytes) are isolated from 10ml of Heparine-blood (Vacutainer) from IDDM patients or controls with Ficoll-Hypaque (Pharmacia).

5×10^6 PBL are incubated with or without 10^3 U/ml recombinant human INF- γ (Gibco-BRL) for 48 hours.

100 μ g/ml Mitomycin C (Calbiochem) are added to inactivate for 10^7 cells for 1 hour at 37°C, and extensive washing is performed.

Culture with T cell hybridomas bearing human V β -2, -3, -7, -8, -9, -13 and -17 at stimulator : responder ratios of 1 : 1 and 1 : 3 in 96 round bottom wells.

TCR-crosslinking with anti-CD3 antibodies (OKT3) is used as a positive control for each individual T hybridoma.

IL-2 release into the supernatant is measured with the indicator cell line CTLL2 according to standard procedures.

Results are expressed as percentage of maximal stimulation obtained with TCR crosslinking in the same experiments.

A selectively induced TCR-crosslinking and IL-release of V β 7 is interpreted as being compatible with the presence of IDDM-SAG in PBL from the individual analysed.

EXAMPLES

In two patients with type I diabetes, a dominant pancreatic enrichment of one Vb-family, Vb 7, has been observed (Conrad et al., 1994). The same dominant enrichment of Vb 7 could be mimicked by stimulating T cells of diverse haplotypes with surface membrane preparations derived from the pancreatic inflammatory lesions but not with membranes from MHC-matched healthy control islets. This was taken as evidence for the presence of a surface membrane-associated SAG (Conrad et al., 1994).

In the framework of the present invention, the hypothesis that this SAG is of endogenous retroviral origin has been tested. Below it is shown that the SAG identified in these two patients is encoded by a human endogenous retrovirus related to MMTV. Expression of this endogenous SAG in IDDM suggests a general model according to which self SAG-driven and systemic activation of autoreactive T cells leads to organ-specific autoimmune disease.

Example 1. Cultured leukocytes from inflammatory b-cell lesions of IDDM-patients release Reverse Transcriptase activity

Expression of cellular retroelements may be associated with measurable Reverse Transcriptase-activity (RT) (Heidmann et al., 1991). An RT-assay detected up to a hundredfold increase in RT-activity in supernatants

from short-term cultures of freshly isolated pancreatic islets derived from two patients (Figure 1A), (Conrad et al., 1994; Pyra et al., 1994). No RT-activity above background levels was detected in medium controls, indicating that the RT-activity could not be accounted for by a contamination of the synthetic media and sera with animal retroviruses. We can also exclude the possibility that the RT-activity represents cellular polymerases released into the supernatant by dying cells. Indeed, no RT-activity can be detected in cultures from non-diabetic controls under conditions in which cell death is strongly enhanced, namely mitogen treated peripheral blood lymphocytes (PBL), splenocytes and cocultures of islets with allogeneic T cells. Moreover, the IDDM-derived islets were cultured for 5 days, whereas control cultures were sequentially analysed for up to 4 weeks. Finally the absence of RT-activity in the supernatants of the mitogen-treated control PBL also excluded the possibility that the RT-activity detected with the IDDM islets was simply due to non-specific cell activation. Both, the islets and the inflammatory infiltration represented potential sources for the enzymatic activity. As shown in Figure 1B, supernatants from cultured spleen cells from the patients contained more RT-activity than the inflammatory b-cell lesions. Moreover, the RT-activity disappeared together with the local inflammatory lesion in two patients with chronic and long-standing disease, but it persisted in cultured spleen cells from the same patient (Figure 1B). This was interpreted as being

compatible with the leukocytes as the most likely source of this RT-activity.

Example 2. Isolation of a full length retroviral genome, IDDMK_{1,2}22, from supernatants of IDDM islets

A strategy to isolate putative retroviral genomes from polyadenylated RNA extracted from the supernatants of IDDM islets was developed (Figure 2A). This strategy relies on the following three characteristic features of functional retroviruses. First, retroviral genomes contain a primer binding site (PBS) near their 5' end. Cellular tRNAs anneal to the PBS and serve as primers for Reverse Transcriptase (reviewed by Whitcomb and Hughes, 1992). Second, the R (repeat) sequence is repeated at the 5' and 3' ends of the viral RNA (Temin, 1981). Third, the RT-RNase H region of the pol gene is the most conserved sequence among different retroelements (McClure et al., 1988; Xiong and Eickbusch, 1990). These three features were exploited in a six step procedure as follows.

1) To isolate the 5' ends (5'R-U5) of putative retroviral RNA genomes, a 5' RACE procedure was performed with primers complementary to known PBS sequences (cPBS primers) (Weissmahr et al., 1997). Most retroviruses known have a primer binding site (PBS) complementary to one of only four individual 3' ends of tRNAs : tRNA^{Pro}, tRNA^{Lys3}, tRNA^{Lys1.2} and tRNA^{Trp}. Accordingly, sequence-specific primers complementary to

the four PBSs were used to derive cDNA (Weissmahr, 1995). The amplification products resulting from anchored PCR and of 100 - 700 bp in size were sequenced and analyzed for the presence of consensus sequences typically found in retroviral 5' R-U5s (Weissmahr, 1995).

Eight different candidate 5'R-U5 sequences (5'K_{1,2}-1, -4, -10, -16, -17, -22, -26 and -27) were obtained with the cPBS-Lysine_{1,2} primer. All eight sequences contained features typical of the 5' ends of retroviral genomes (Temin, 1981). These include the presence at the expected positions of i) a PBS region, ii) conserved and correctly spaced upstream regulatory sequences, such as a poly(A) addition signal and site, and the downstream GT- or T - rich elements (Wahle and Keller, 1996), iii) a putative 5' end specific U5 region and iv) a putative R region. Of the eight 5' R-U5 sequences isolated, three (5'K_{1,2}-1, -4, and -22) were identified on the basis of sequence homology as belonging to previously identified families of human endogenous retroviruses (HERVs) that are closely related to mouse mammary tumour viruses (MMTV), namely HERV-K(C4) (Tassabehji et al., 1994), HERV-K10 and HERV-K18 (Ono, 1986a; Ono et al., 1986b). The remaining five sequences exhibited only a distant relationship with HERV-K retroviruses.

2) A repeat (R) region conserved in the 5' R-U5 and the 3' U3-R-poly(A) is essential for retroviral first strand DNA synthesis to proceed to completion (Whitcomb and Hughes, 1992). Primers specific for the R

region-sequence obtained for individual 5' R-U5s were used to prime the cDNA synthesized with oligo(dT), (Weissmahr, 1995). Products resulting from anchored PCR were sequenced and analyzed for the presence of a conserved R region followed by a poly(A)-tail. The eight 3'R-poly(A) ends (3'K_{1,2}-1, -4, -10, -16, -17, -22, -26 and -27) corresponding to the eight different 5'R-U5 regions identified in step 1 were isolated by means of a 3' RACE procedure using primers specific for the R regions. In each case, the isolated sequences contained the expected R region followed by a poly(A) tail.

3) The conserved RT-RNase H region within the *pol* gene was next amplified by PCR using degenerate primers (Medstrand and Blomberg, 1993). 15 individual subclones were sequenced and all exhibited approximately 95% similarity at the protein level to the RT-RNase H region of the HERV-K family.

4) The 5' moiety (from the U5 region at the 5' end to the *pol* gene) of the putative retroviral genome was amplified by PCR using primers specific for the eight different U5 regions present in the 5'R-U5 sequences (isolate in step 1) in conjunction with a primer specific for the 3' end of the central *pol* region (isolated in step 3). The expected size of the PCR product corresponding to the 5' moiety of full length HERV-K retroviruses is 3.6 kb (Ono et al., 1986b). Only the PCR reaction using the primer specific for the K_{1,22} 5' end clone consistently yielded a fragment of this size. Sequence analysis of several independent

clones confirmed that this 3.6 kb fragment contains the R-U5-PBS region followed by coding regions corresponding to the *gag* and *pol* genes, and thus indeed represents the 5' moiety of an intact retroviral genome.

5) The 3' moiety (from the *pol* gene to the 3' end) of the putative retroviral genome was amplified by PCR using a primer specific for the 5' end of the central *pol* region (isolated in step 3) and primers specific for the poly(A) signals present in the 3'R-poly(A) sequences (isolated in step 2). The expected size of the PCR product corresponding to the 3' moiety of full length HERV-K-retroviruses is 5 kb (Ono et al., 1986b). The PCR reaction using a primer specific for the 3' end clone K_{1,2}22, which is the one that should correspond to the 3' end of the retrovirus from which the 3.6 kb 5' moiety was amplified in step 4, consistently yielded a fragment potentially representing an intact 3' moiety of 5 kb. Sequence analysis of several independent clones confirmed that this 5 kb fragment indeed contains coding regions corresponding to the *pol* and *env* genes followed by the expected U3-R-poly(A) region.

6) Finally, the presence of an intact 8.6 kb retroviral genome containing the overlapping 5' and 3' moieties isolated in steps 4 and 5 was confirmed by PCR using primers specific for its predicted U5 and U3 regions.

The full length retroviral genome that was isolated was called IDDMK_{1,2}22, where IDDM refers to the tissue source, K_{1,2} refers to Lysine_{1,2} cPBS primer and

22 represents the serial number of the clone. IDDMK_{1,22} was determined to be novel retrovirus on the basis of two criteria. First, it has a unique pattern of restriction enzyme cleavage sites that is distinct from that of other known viruses. Second, its nucleotide and amino acid sequences in non-coding and coding regions diverge from other known retroviruses by at least 5-10 %.

IDDMK_{1,22} was the only full length virus identified in these experiments, suggesting that it is the only functional retrovirus specifically associated with the supernatants of the cultured IDDM islets. PCR reactions using primers specific for the other 5'R-U5-PBS and 3'U3-R-poly(A) clones isolated in steps 1 and 2 did not yield fragments of the size expected for intact retroviral genomes in steps 4 and 5. In particular, primers specific for the 5' and 3' ends corresponding to the ubiquitous HERV-K10 virus did not amplify fragments corresponding to complete genomes, although this virus is known to be released as full length genome associated with viral particles from several cell lines and tissues (Tönjes et al., 1996). Our inability to detect full length HERV-K10 genomes in the IDDM islet supernatant is unlikely to be due to a technical problem because it could be amplified very efficiently from both genomic DNA and a size selected cDNA library prepared from a B-lymphoblastoid cell line (data not shown). It is more likely that HERV-K10 is not released in significant amounts by the cultured IDDM islets.

Finally, i) we confirmed by RNA-specific PCR that sequences identical, or highly similar, to the 3' U3-R-poly(A) of IDDMK_{1,2} were present in RT-positive but not in RT-negative samples analysed; ii) in a preliminary epidemiological study we detected by PCR sequences identical, or highly similar, to the 3' U3-R-poly(A) of IDDMK_{1,2} only in the plasma of 10 recent onset IDDM patients but not in the plasma of 10 age-matched non diabetic controls (Figure 2F); and iii) we confirmed by PCR the presence of sequences identical, or highly similar to the U3-R region of IDDMK_{1,2} in genomic DNA of IDDM patients (n = 10) and non diabetic controls (n = 10) (Figure 2F). In summary, these data indicate that IDDMK_{1,2} is an endogenous retrovirus that is released from leukocytes in IDDM patients but not in non diabetic controls.

Example 3. IDDMK_{1,222} is a novel member of the MMTV-related family of HERV-K, and is related to HERV-K10

To evaluate the relationship between IDDMK_{1,222} and other known retroviruses we derived phylogenetic trees for subregions exhibiting different degrees of conservation (Galtier et al., 1996; Saitou and Nei, 1987; Thompson et al., 1994). The three regions chosen for this analysis were the RT region of the *pol* gene (Figure 3B), the outer region (SU, surface) of the *env* gene (Figure 3A) and the U3 region of the LTR (Figure

3C). The RT and SU regions were selected to construct interspecies phylogenetic trees because they represent, respectively, the most highly conserved and the most variable of the protein coding regions (McClure et al., 1988). The U3 region of the LTR was chosen to construct an intraspecies tree of the family to which IDDMK_{1,222} belongs because LTR sequences are conserved in size and sequence only within a given species, and the U3 region accounts for most of the intraspecies differences (Temin, 1981). As shown in Figure 3A, the ENV polyprotein of IDDMK_{1,222} is most closely related to that of HERV-K10. Both proteins are related to those of MMTV and Jaagsiekte sheep retrovirus (JSRV). The same is essentially true for the RT-subregion of the POL polyprotein, where IDDMK_{1,222} and HERVK10 are most closely related to the B-type retrovirus MMTV (Figure 3B). Figure 3C illustrates, that K_{1,21} is related to HERV-K(C4), while K_{1,24} and IDDMK_{1,222} are related to the K10/K18 subfamily. Within this family, K_{1,24} is closely related to K10, whereas IDDMK_{1,222} appears to be more distant.

Example 4. IDDMK_{1,222} encodes a V β 7-specific SAG

The strategy used to identify a putative SAG-function encoded by IDDMK_{1,222} was dictated by 1) predictions based on the biology of the MMTV-SAG, 2) general requirements for a protein-protein interaction

between a SAG and MHC class II molecules and 3) intracellular trafficking mechanisms used by proteins encoded by retroviruses. The prototypical retroviral SAG of MMTV is a type II transmembrane protein that is encoded within the U3 of the 3' LTR (reviewed by Acha-Orbea and McDonald, 1995). It is targeted into the MHC class II peptide loading compartment and exported to the cell surface. On the basis of potential splice donor (SD) and acceptor sites (SA) present in its sequence, IDDMK_{1,222} is expected to generate two subgenomic mRNAs, one encoding ENV and a second transcript comprising the U3-R region (Figure 4A). Based on these criteria we produced an episomal expression construct (pPOL-ENV-U3) with a 5' SD positioned upstream of the truncated pol, env and U3-regions (Figure 4A). It is expected that both of the putative subgenomic mRNAs can be generated from this construct (Figure 4A).

Retroviral- and control-transfectants of monocyte- and B lymphocyte-cell lines were generated and tested for their ability to stimulate MHC compatible and allogeneic T cell lines in a V β 7-specific manner. Monocytes do not express measurable MHC class II surface proteins in the absence of induction by Interferon- γ (INF- γ); the MHC class II transactivator CIITA mediates INF- γ -inducible MHC class II expression (reviewed by Mach et al., 1996). As shown in Figure 4A, transient monocyte (THP1, U937) transfectants induced with INF-g and expressing the truncated IDDMK_{1,222} genome (pPOL-ENV-U3) stimulated in

a dose-dependent fashion T cell lines from MHC-compatible donors essentially to the same extent. The mitogenic effect was dependent on the presence of MHC class II, since INF-g-mediated MHC class II expression specifically induced the stimulatory capacity of retroviral- as compared to control-transfectants (Figure 4B). The use of THP1 cells rendered constitutively MHC class II positive by transfection with CIITA resulted in a stimulation comparable to INF-g-induction, suggesting that the INF-g-induced and CIITA-dependent MHC class II expression was indeed responsible for this functional difference (Figure 4C). The mitogenic effect is not MHC-restricted, since a response exceeding allostimulation was observed when PBL from several different MHC-disparate donors were tested for proliferative responses to monocytes transfected with pPOL-ENV-U3 (Figure 4D). In essence, these functional data suggest that the truncated IDDMK_{1,222} (pPOL-ENV-U3) genome is responsible for a mitogenic effect that is MHC class II-dependent but not MHC-restricted.

Experiments were performed in bulk-cultures using TCR-V β -specific stimulation and expansion as a readout. Retroviral THP1 transfectants induce a more than 15 fold increase in the number of the V β -7 family but not of the two control families tested (V β 8, V β 12) after specific stimulation and subsequent amplification (Figure 5, Table 1). This was verified by using two different V β -7-specific monoclonal antibodies, 3G5 and 20E. A comparable effect was also observed when PBL

from MHC-disparate donors were tested. This was interpreted as evidence for the presence a V β -7-specific SAG.

The monocytic cell lines were at least 3 times more efficient in terms of specific TCR Vb-7 amplification as compared to the most efficient B lymphoblastoid cell line (Table 1). This difference could not be explained by variations in the level of MHC class II expression or by the individual MHC haplotypes present. On the other hand, it may be due to differential expression of costimulatory molecules or secretion of cytokines. In conclusion, by all criteria known to date, IDDMK_{1,222} encodes a mitogenic activity having all features of a Vb-7-specific SAG.

TABLE 1 : IDDMK_{1,222} mediates a V β 7-specific SAG-effect

TRANSFECTANT	V β -FAMILY		
	V β -7	V β -8	V β -12
Raji-pPOL-ENV-U3	7%	5%	2.5%
Raji-pVECTOR	1.5%	5.5%	2%
THP1-pPOL-ENV-U3	16%	5.3%	2.8%
THP1-pVECTOR	1%	5.8%	3%

Example 5. The SAG function is mediated by the N-terminal moiety of the env protein

A series of deletional mutants were generated that contained either the truncated *pol-env-U3* region (pPOL-ENV-U3), the truncated *pol* gene alone (pPOL), or the truncated *pol* gene followed by the *env* gene truncated downstream of the premature stop codon found in all clones (pPOL-ENV/TR), (Figure 6A). In addition, a C-terminally truncated *env* gene was generated as an individual expression unit (pCI-ENV/TR). As shown in Figure 6B, by excluding the *env*-coding region the SAG-function is selectively lost (pPOL). If, however, the truncated *env* gene is included (pPOL-ENV/TR), the stimulatory capacity is restored to levels comparable to pPOL-ENV-U3. In addition, expression of the truncated *env* gene alone (pCI-ENV/TR) is sufficient for function. These findings demonstrate that the SAG function is mediated by the N-terminal moiety of the *env* gene comprising 153 amino acids. The nucleotide and predicted amino acid sequences of the minimal stimulatory region are shown in Figure 7. As shown in Figure 3A, this predicted protein resembles the N-terminal ENV proteins of related HERVs (HERV-K10), and those of the B-type retroviruses (MMTV, JSRV). However, there is no significant sequence homology with either MMTV-SAG, other SAGs, or autoantigens known to be important in IDDM.

Here, evidence is provided showing that a human endogenous retrovirus, IDDMK_{1,222}, is released from leukocytes in patients with acute onset type I diabetes. In preliminary experiments IDDMK_{1,222} RNA sequences were detectable in the plasma of IDDM patients at disease onset but not in the plasma of age-matched healthy controls. This novel human retrovirus is related to MMTV and encodes a SAG with functional characteristics similar to the one encoded by MMTV. In contrast to MMTV, however the IDDM-associated SAG is encoded within the retroviral *env* gene rather than within the 3' LTR. It has the same TCR V β 7-specificity with the SAG originally identified in the IDDM patients. This SAG is thus likely to be the cause of the V β 7-enriched repertoire of islet-infiltrating T lymphocytes.

IDDMK_{1,222} as a member of the HERV-K class of endogenous retroviruses

HERV-K genomes exist in two different forms, type I genomes which are largely splice deficient and type II genomes which generate three subgenomic mRNAs (Tönjes et al., 1996; Ono, 1986). A 292 bp insert at the *pol-env* boundary with clustered nucleotide changes downstream of the splice acceptor site are present in type II but not in type I genomes (Tönjes et al., 1996). The insert affects both, the *env* and *pol* gene:

- i) type II genomes have a stop codon between *env* and

pol which is missing in type I genomes and ii) have a considerably longer N terminal *env* region. The 292 bp insert and the clustered nucleotide changes have been proposed to be responsible for the efficient splicing of type II genomes (Tönjes et al., 1996). IDDMK_{1,222} is missing the 292 bp insert but has two in frame stop codons between *env* and *pol* and the clustered nucleotide changes downstream of the SA typical of those found in type II genomes. In terms of splice efficiency, IDDMK_{1,222} may be in an intermediate position between type I and II genomes. This and the altered N terminal sequences in IDDMK_{1,222} with respect to type II genomes may affect SAG expression in vivo. However, as shown in Figure 4, the 3' terminal moiety (POL-ENV-U3) of the IDDMK_{1,222} genome mediates the SAG function in vitro. Moreover, it is known from MMTV that the SAG function in vivo may be present at levels where the respective protein remains undetectable (Winslow et al., 1992; reviewed by Acha-Orbea and MacDonald, 1995).

The model: human self SAGs as activators of autoreactive T cells in type I diabetes

A model is proposed according to which induction of self SAGs in systemic and professional APCs, outside the pancreas, leads to autoimmunity in genetically susceptible individuals. The model implies two steps, the first is systemic, the second organ-specific. The initial event is a systemic, polyclonal activation of a

Vb-restricted T cell subset, triggered by the expression of an endogenous retroviral SAG in professional MHC class II⁺APCs. In a second step, autoreactive T cells within the subset of SAG-activated T lymphocytes initiate organ-specific tissue destruction. The evidence presented here, however, does not rule out that the release of the IDDMK_{1,222} RNA sequences in vivo and the SAG function associated with IDDM in these patients are the consequence rather than the cause of the inflammation.

The expression of self SAGs can in principle be modulated by two variables: physiological endogenous stimuli or environmental stimuli. A possible physiological stimulus might be steroid hormones. HERV-K10 expression is steroid-inducible in vitro and this is possibly the result of hormone response elements (HRE) present in its LTR (Ono et al., 1987). IDDMK_{1,222} and HERV-K10 share the same putative HRE in their respective LTRs (Ono et al., 1987), (Figure 3). Steroid inducibility of IDDMK_{1,222} could therefore also occur in vivo, in analogy to the well documented example of the transcriptional control by steroid hormones of the MMTV promoter (reviewed by Acha-Orbea and Mac Donald, 1995). Infectious agents are of major importance when considering environmental factors. Examples include the cellular SAGs that are expressed by herpesvirus-infected monocytes and B-lymphocytes (Dobrescu et al., 1995; Sutkowski et al., 1996). In both cases, HERVs have not been excluded as a potential source of the SAG-activity. It is thus conceivable that SAGs are

being selectively expressed in response to ubiquitous pathogens such as herpesviridae (reviewed by Roizman, 1996). In fact, HERVs are induced by a variety of environmental stresses, and some of them behave as hepatic acute-phase genes (reviewed by Wilkinson et al., 1994).

The experimental evidence presented suggests that the RT-activity, the IDDMK_{1,222} RNA sequences and in consequence the SAG may derive from leukocytes rather than from the pancreatic b-cells. This may indicate that expression of the retroviral SAG is induced preferentially in systemically circulating professional MHC class II⁺ APCs. The highest rate of IDDM coincides with puberty (10-14 years) in both sexes (Bruno et al., 1993). Infections with ubiquitous viruses (reviewed by Roizman, 1996) may act synergistically with an increase in the circulating levels of steroids to enhance expression of the SAG in professional APCs. Autoreactive T cells can be readily demonstrated in the mature repertoire of healthy individuals (Pette et al., 1990). However, in order to be able to migrate to the target tissue these T cells have to be activated (reviewed by Steinman, 1995). These considerations lead us to the hypothesis that among the Vb7⁺-T cells activated by IDDMK_{1,222}-SAG, some are autoreactive and migrate to the target tissue where b-cell specific death ensues. Once b-cells die, cellular antigens are liberated and the immune response perpetuated through determinant spreading (reviewed by McDevitt, 1996).

The concept of IDDMK_{1,222}-sag as autoimmune gene

Known genes conferring susceptibility to autoimmune diseases are host-derived, stably inherited Mendelian traits and contribute in a cumulative fashion to the familial clustering of the disease without causing disease per se (reviewed by Todd, 1996). IDDMK_{1,222} should be viewed as mobile genetic element with the potential to move within the host genome due to multiple mechanisms, including retrotransposition, homologous recombination, gene conversion and capture, resulting in multiple copies of individual HERVs (reviewed by Preston and Dougherty, 1996; Wain-Hobson, 1996). This renders family studies dealing with searches for HERV-disease association difficult. It should be noted, however, that there is little or no plus / minus genetic polymorphism in different humans at the HERV-K loci and as yet no evidence for mobility. Interestingly, an IDDMK_{1,222}-related HLA-DQ-LTR is associated with susceptibility to IDDM, possibly due to cosegregation with the HLA (Figure 3C), (Badenhoop et al., 1996). In addition, infectious transmission cannot be excluded, as is the case for two closely related virus groups containing endogenous and exogenous variants: MMTV and JSRV (Figure 4A and 4B), (reviewed by Acha-Orbea and McDonald, 1995; York et al., 1992).

In summary, this candidate autoimmune-gene has distinctly different features from classical, disease-

associated susceptibility genes. It has the potential of being transmitted as either an inherited trait or as an infectious agent. Moreover, this gene has no apparent essential function for the host but it may have instead an inducible and intriguing potential to directly cause disease whenever expressed in genetically susceptible individuals.

Example 6. Development of an animal model to document and study the Sag effect in vivo

Several mouse cell lines, in particular a B lymphocytes line (A20) and a monocyte line (WEHI-3) were stably transfected with the IDDM Sag cDNA (corresponding to the minimal region encoding a.a. 1 to 153 of the env protein of IDDM1,2,22, as described above). The B cell lines express mouse MHC class II molecules constitutively. In the case of monocyte lines, the transfectants are induced to express mouse MHC class II molecules by treatment with mouse interferon gamma (100-1000 units of mouse interferon (Genzyme) per ml for 48 hrs).

These MHC class II positive Sag transfectants were capable of stimulating (in vitro) human T lymphocytes of the V β 7 specificity, and not V β 8 or V β 12 as negative controls. This demonstrates that the IDDM Sag can function when expressed on MHC class II positive mouse cells. These Sag-expressing, MHC class II positive, mouse transfectants are used to immunise mice against the Sag protein and to generate anti Sag

monoclonal antibodies, using as control the homologous untransfected cell lines.

This Sag effect lead to the stimulation of V β 7-specific T lymphocytes of both the CD4 and the CD8 type. This observation indicates that the IDDM Sag functions in T cell activation in a manner that is independent of the co-receptors CD4 and CD8. This situation is different from what is observed in the case of the mouse MMTV Sag, where only CD4 T lymphocytes are stimulated.

The same MHC class II positive mouse stable Sag transfectants (A 20, B lymphocytes and WEHI-3, monocytes), expressing the minimal functional region of IDDM Sag defined above (and corresponding to a.a. 1 to 153 of the env protein of IDDM1,2,22) specifically stimulated mouse T lymphocytes of the V β 4 and the V β 10 specificity. (These are the most highly related mouse Vb sequences, from a structural point of view, to human V β 7).

Again, both CD4 and CD8 mouse T lymphocytes were activated, indicating a Sag mediated activation that is independent of the CD4 and CD8 co-receptors.

More importantly, injection of the same stable Sag transfectants into mice (either in the hind foot path or in the tail vein) lead to in vivo activation of T lymphocytes, again with the same V β specificity observed upon in vitro mouse T cell activation by the IDDM Sag. T cell activation and V β specificity in response to the injection of Sag transfectants was

monitored by analysis of T lymphocytes in draining lymph nodes and in the spleen.

The ability to induce V β -specific T lymphocyte activation in vivo in mice following injection of MHC class II positive transfectants expressing IDDM Sag indicates that the biological effect of IDDM Sag can now be monitored in an in vivo animal model. This allows the testing in vivo, not only of a Sag biological effect, but also of potential inhibitors of the effect of Sag, such as anti-Sag antibodies, including monoclonal anti-Sag antibodies, and small molecular weight inhibitors of Sag (first identified as inhibitors of Sag in in vitro cell based assays). Finally, this in vivo model of the biological effect of Sag allows to test the effect of prior immunisation of animals with the Sag protein (or derivatives thereof) on the biological effect of Sag in vivo. This model provides a test of the possibility of a protective vaccination against IDDM Sag in vivo.

Transgenic mice carrying the IDDM Sag gene have been obtained. The Sag gene is under the control of a tetracycline operator element (consisting of a heptameric repeat of the Tn motive linked to a minimal promoter). These transgenic mice have been crossed with two other transgenic mice carrying the tetracycline transactivator gene (TTA) under the control of the CMV promoter. One transgenic (CMV-TTA) induces the tet transactivator upon withdrawal of tetracycline, while the other (CMV-RTTA) induces the tet transactivator in the presence of tetracycline. These double transgenic

mice permit the deliberate, selective and controlled expression of Sag in vivo, allowing the subsequent study of immunopathological consequences of Sag expression.

Exactly the same steps can be followed (= Sag-expressing mouse cells and Sag expression in vivo) to establish animal models of the effect of other Sags encoded by other HERVs in the context of other autoimmune diseases, such as multiple sclerosis or rheumatoid arthritis.

Experimental Procedures

Patients

The the islets and spleens from patients with acute onset- and chronic IDDM and non diabetic organ donors were provided by the Pittsburgh Transplant Institute (Conrad et al., 1994).

The plasma and genomic DNA from patients and controls for the epidemiological study were isolated by the Diabetes Register in Turin, Italy (Bruno et al., 1993). The samples were collected within 1 month after the clinical diagnosis from patients, aged from 0 - 29 years (Bruno et al., 1993).

RT assays

RT assays were performed as described (Pyra et al., 1994).

Isolation of full length retroviral genomes

A description of the criteria used to identify unknown retroviral 5' R-U5s and 3' R-poly(As) has been published (Weissmahr et al., 1997).

I. Primers sequences for the 3'moiety of the putative retroviral genomes; abbreviations are according to Eur. J. Biochem. (1985). 150, 1-5.

A. RT region

RT 1a 5'YAAATggMgWAYgYTAACAgACT3'

RT 1b 5'YAAATggMgWAYgYTAAGTgACT3'

RT 2a-nested

5'CgTCTAgAgCCYTCTCCggCYATgATCCCg3'

RT 2b-nested

5'CgTCTAgAgCCYTCTCCggCYATgATCCCA3'

B. 3' U3-R-Poly(As): all primers have an identical 5'-anchor:

5'TgCgCCAgCAATgTATCCATg3'+ sequence-specific part

#1K1,2-1 5'gggTggCAGTgCATCATAggT3'

#4K1,2-4 5'gggAgAgggTCAGCAGCAgACA3'

#K1,2-10 5'gACAgCAAgCCAgTgATAAgCA3'

#K1,2-16 5'ggAACAgggACTCTCTgCA3'

#K1,2-17 5'gggAAgggTAAggAAgTgTg3'

#K1,2-22 5'ggTgTTTCTCCTgAgggAg3'

#K1,2-26 5'gAAgAATggCCAACAgAAgCT3'

#K1,2-27 5'gggAAACAaggAgTgTgAgT3'

common, secondary anchor primer:

3' U3-R-poly(As) common

5' CATgTATATgCggCCgCTgCgCCAgCAATgTATCC
ATgg3'

II. Primer sequences for the 5' moiety of the genome:

A. RT-region

RT 1 5' TATCTTTCgTTTCTgCAgCAC3'
RT 2 5' TAACTggTTgAAgAgCTCgACC3'

B. 5'-R-U5

R-U5-1 5' ATACTAAgggggACTCAgAggC3'
R-U5-2 5' CAgAggCTggTgggATCCTCCATATgC3'

The PCR conditions were as follows: 1x 94° C 2 min; 45° C 5 min; 68° C 30 min; 10x 94° C 15 sec; 45° C 30 sec + 1° C/cycle; 68° C 3 min 30 sec; 25x: 94° C 15 sec; 55° C 30 sec; 68° C 3 min 30 sec + 20 sec/cycle. Primers were used at 300 nM final concentration, dNTPs at 200mM, with 52 U/ml of Taq-Pwo polymerase-mix (Boehringer Mannheim). One vol% of first-round PCR was subjected to a nested PCR. Size selected and purified amplification products were blunted, EcoRI adapted and subcloned into EcoRI-digested lZAPII-arms. After two rounds of hybridisation 20 individual clones were rescued as plasmids. Eleven clones were selected for further analysis based on a conserved restriction pattern. An equivalent procedure was followed for the 5' moiety of the genome. Sequencing was performed on an

automatic sequencer (ABI, Perkin Elmer) using subgenomic clones.

Epidemiological study. RNA-PCR. Three ml of blood was collected in EDTA tubes (Vacutainer) and further processed within 6 hours. Samples were subjected twice to centrifugation, for 4×10^3 G, 10 min at 4°C. Total RNA was extracted from 560 ml of plasma (QIAamp; Qiagen). Four vol % of total RNA was used for a single tube RT-PCR using thermostable AMV, Taq and Pwo (Boehringer Mannheim). Reactions contained at a final concentration: di-Na salts of dNTPs at 0.2 mM; DTT at 5 mM; 10 U recombinant RNAsin (Promega); 1.5 mM MgCl₂; R-poly(A) primer 5' TTT TTg AgT CCC CTT AgT ATT TAT T 3'; U3 primer 5' Agg TAT TgT CCA Agg TTT CTC C 3', both at 0.3 mM. RT was performed at 50°C for 30 min directly followed by 94° C 2 min; 94° C 30 sec, 68° C 30 sec, - 1.3° C each cycle, 68° C 45 sec for a total of 10 cycles; 94° C 30 sec, 55° C 30 sec, 68° C 45 sec for a total of 25 cycles. The amplified material (487 bp) was subjected to agarose gel electrophoresis followed by alkaline transfer and hybridisation with probes generated from the IDDMK_{1,222} U3-R-region. Genomic PCR. 100 ng of genomic DNA was subjected to PCR. Reactions contained at a final concentration: dNTPs at 200 mM; 1.5 mM MgCl₂; 2.6 U of Taq-Pwo (Boehringer Mannheim); U3-primer 5' Agg TAT TgT CCA Agg TTT CTC C 3'; R-primers either 5' CTT TAC AAA gCA gTA TTg CTg C 3, or 5' gTA AAg gAT CAA gTg CTg TgC 3' at 300 nM. The amplified products were 300 and 395 bp in size,

respectively. The cycling profile was as follows: 94° C 2 min; 94° C 15 sec, 68° C 30 sec, -1.3° C each cycle, 72° C 45 sec for a total of 10 cycles; 94° C 15 sec, 55° C 30 sec, 72° C 45 sec for a total of 25 cycles.

Sequence alignment and phylogenetic trees

Sequences were aligned with CLUSTAL W (Thompson et al., 1994). Alignments were checked and manually corrected with the SEA VIEW multiple sequence alignment editor (Galtier et al., 1996). Phylogenetic trees were computed from multiple alignments using the "neighbour joining" method (Saitou and Nei, 1987).

Expression

Constructs. pPOL-ENV-U3: a SacI-NotI fragment derived from 11 IDDMK_{1,222} clones was ligated with 1) a BamHI-SacI adapter containing a consensus SD and 2) with a NotI-XbaI adapter and 3) was subcloned into BamHI-XbaI digested pLDR2-arms, selected for by two rounds of screening and plasmids rescued. At least five independent clones were used for transfections. pPOL: pPOL-ENV-U3 was digested with KpnI-NotI, blunted and religated. pPOL-ENV/TR: a stimulatory clone was digested with XbaI and religated. pCI-ENV/TR: 1 ng of pPOL-ENV-U3 was amplified with the primers 5' gAC TAA gCT TAA gAA CCC ATC AgA gAT gC 3' and 5' AgA CTg gAT

CCg TTA AgT CgC TAT CgA CAg C 3'. The amplified products were subcloned into pCI-neo (Promega).

Cells and cell lines. Monocytic cell lines: THP1, U937. B-lymphoblastoid cell lines: Raji, BOLETH, SCHU and WT 51. T cells of molecularly MHC-typed blood donors were generated by positive selection with anti-CD3 coated immunomagnetic beads (Milan-Analytika).

Transfections. Transient transfectants were used for functional assays 48 hours after transfection; stable transfectants were selected for 2 weeks in progressive concentration of Hygromycin B to a final concentration of 250 mg/ml for lymphoblastoid lines, and 50 mg/ml for monocytic cell lines.

Functional assays. Transfectants were treated with Mitomycin C (Calbiochem) at 100 mg/ml per 10^7 cells for 1 hour at 37° C and washed extensively. Proliferation assays. 10^5 CD3-beads-selected, MHC compatible T cells or Ficoll-Paque-isolated allogeneic PBL were cultured with transfectants at stimulator: responder ratios of 1:1; 1:3 and 1:10 for 48 and 72 hours in 96 round-bottom wells at 37° C. ^3H -Thymidine was then added at 1mCi/well and incorporation measured after 18 hours incubation at 37° C. FACS analysis and antibodies used were as described; after 3 days of specific stimulation, at T: non-T ratios of 1:1 for syngeneic, and 10:3 for allogeneic stimulations, the T cells were further expanded in 20 U/ml recombinant IL-2 for 6 days before flow cytometric analysis (Conrad et al., 1994).

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CLAIMS

1. Process for the diagnosis of a human autoimmune disease, including pre-symptomatic diagnosis, said human autoimmune disease being associated with human endogenous retrovirus (HERV) having Superantigen (SAg) activity, comprising specifically detecting in a biological sample of human origin at least one of the following :

- I- the mRNA of an expressed human endogenous retrovirus having Superantigen (SAg) activity, or fragments of such expressed retroviral mRNA, said retrovirus being associated with a given autoimmune disease, or
- II- protein or peptide expressed by said retrovirus, or
- III- antibodies specific to the proteins expressed by said endogenous retrovirus, or
- IV- SAg activity specifically associated with said endogenous retrovirus,

detection of any of the species (I) to (IV) indicating presence of autoimmune disease or imminent onset of autoimmune disease.

2. Process according to claim 1 wherein the expressed retroviral mRNA is specifically detected by nucleic acid amplification using primers, one of which is specific for the poly(A) signals present in the 3' R-poly(A) sequences at the 3' extremity of the retrovirus.

3. Process according to claim 1 wherein the protein or peptide expressed by the endogenous retrovirus is detected using antibodies specific for the said retroviral protein or peptide.

4. Process according to claim 1 wherein the antibodies specific to retroviral protein are detected by use of the retroviral protein, or fragments thereof with which the antibodies specifically react.

5. Process according to claim 1 wherein SAg activity specifically associated with said HERV is detected, the biological sample being a biological fluid containing MHC Class II⁺ cells or cells induced to express MHC Class II molecules, this sample being contacted with cells bearing one or more variable (V)- β T-cell receptor chains, and detecting preferential proliferation of the V β subset, or one of the V β subsets characteristic of said autoimmune disease.

6. Process according to claim 1 wherein the autoimmune disease is type I diabetes and the associated retrovirus having SAg activity is IDDMK_{1,2} 22 comprising the 5' long terminal repeat shown in Figure 7A, the 3' short terminal repeat shown in Figure 7B, or the env encoding sequences shown in Figure 7C, Figure 7D or Figure 7E, or variants thereof presenting approximately at least 90% sequence identity.

7. Process according to claim 6 wherein the expressed retroviral RNA is specifically detected by nucleic acid amplification using primers, one of which is specific for the poly(A) signals present in the 3' R-poly(A) sequences at the 3' extremity of IDDMK_{1,22}.

8. Process according to claim 7 wherein the poly(A) specific primer is

5' TTTTGGAGTCCCCTTAGTATTTATT 3' or

5' T₍₂₀₎GAGTCCCCTTAGTATTTATT 3'

9. Process according to claim 6 wherein protein expressed by IDDMK_{1,22} is detected, said protein being either the protein encoded by the N-terminal moiety of the env coding region of IDDMK_{1,22} as illustrated in Figure 7D or 7G, or the protein encoded by the pol coding region, as illustrated in Figure 7H, or a protein having at least 90% homology with the illustrated protein, or a fragment of said proteins having at least 6 amino-acids.

10. Process according to claim 6 wherein antibodies specific for env or pol proteins expressed by IDDMK_{1,22} are detected using the env or pol proteins illustrated in Figure 7D, 7G or 7H, or a protein having at least 90% homology with the illustrated protein, or a fragment of said proteins having at least 6 amino-acids.

11. Human endogenous retrovirus having superantigen activity, and being associated with human autoimmune disease, said retrovirus being obtainable from RNA prepared from a biological sample originating from a human autoimmune source, by carrying out the following steps :

i) isolation of the 5' R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3' primer being complementary to known « primer binding sites » (pbs) and the 5' primer being an oligonucleotide anchor ;

ii) isolation of the 3' R-poly(A) ends corresponding to the 5' R-U5 ends, by use of primers specific for the R regions isolated in step i) ;

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region ;

iv) amplification of the 5' moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3' end of the central pol region isolated in step iii) ;

v) amplification of the 3' moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3' R-poly(A) sequences isolated in step ii) ;

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

12. Proviral DNA of a retrovirus according to claim 11.

13. Proviral DNA according to claim 12 obtainable from a biological sample of human origin by :

- i) obtaining retroviral RNA according to the method of claim 11, and further,
- ii) generating a series of DNA probes from the retroviral RNA obtained in i);
- iii) hybridising under stringent conditions, the probes on a genomic human DNA library ;
- iv) isolation of the genomic sequences hybridising with the probes.

14. Nucleic acid molecule comprising fragments of the retroviral RNA or DNA according to any one of claims 11 to 13, said fragment having a length of at least 15 nucleotides and preferably at least 30 nucleotides.

15. Nucleic acid molecule according to claim 14, encoding SAg activity of the retrovirus.

16. Nucleic acid molecule according to claim 15 derived from an endogenous human retrovirus open reading frame and optionally containing at least one internal stop codon.

17. Nucleic acid molecule according to claim 15 or 16 comprising the retroviral env gene.

18. Nucleic acid molecule comprising a sequence complementary to the nucleic acid molecules of any one of claims 11 to 17.

19. Nucleic acid molecule according to claim 18 comprising a ribozyme or antisense molecule to a human retrovirus having SAg activity to a proviral DNA of said retrovirus or a fragment thereof.

20. Nucleic acid molecule capable of hybridizing in stringent conditions, with the nucleic acid molecules of any one of claims 11 to 19.

21. Vector comprising nucleic acid molecules of any one of claims 11 to 20.

22. Nucleic acid molecule comprising at least one of the sequences illustrated in Figures 7A, 7B, 7C, 7D, 7E, or a nucleic acid sequence encoding the POL protein shown in Figure 7H, or a sequence exhibiting at least 90% homology with any of these sequences, or a fragment of any of these sequences having at least 20 nucleotides, and preferably at least 40 nucleotides.

23. Nucleic acid molecule at least partially complementary to any of the sequences according to claim 22.

24. Nucleic acid molecule according to claim 22 comprising a ribozyme or antisense.

25. Nucleic acid molecule which is HERV IDDMK_{1,2-22} comprising each of the sequences illustrated in Figures 7A, 7B, 7C, or sequences having at least 90% identity with these sequences, having a size of approximately 8.5 kb, having SAg activity encoded within the env region illustrated in Figure 7D or 7E, said SAg activity being specific for V β 7 - TCR chains.

26. Protein or peptide having at least 6 amino acids, characterised in that :

- it exhibits SAg activity and optionally is capable of giving rise, directly or indirectly, to autoreactive T-cells targeting tissue characteristic of a given autoimmune disease ;

- it is encoded by a human endogenous retrovirus ;

- it is obtainable from biological samples of patients having autoimmune disease.

27. Protein or peptide according to claim 26, encoded by the env gene of the HERV, or a portion thereof.

28. Protein or peptide according to claim 27 corresponding to a protein or peptide resulting from a

premature translational stop, and/or from a frame shift in the translation of a retroviral open reading frame.

29. Protein or peptide according to any one of claims 26 to 28 obtainable by introducing viral DNA of claim 13 or fragments thereof, or corresponding synthetic DNA into a eukaryotic cell under conditions allowing the DNA to be expressed, and recovering said protein.

30. Protein according to any one of claims 26 to 29 comprising the amino acid sequence shown in Figure 7D, Figure 7F, Figure 7G, Figure 7H, or an amino acid sequence having at least 80% and preferably at least 90 % homology with the illustrated sequences, or a fragment of said sequence having at least 6 amino acids.

31. Antibodies capable of specifically recognising a protein or peptide according to any one of claims 26 to 30.

32. Antibodies according to claim 31 which are monoclonal.

33. Antibodies according to claim 31 or 32 which specifically recognise a HERV protein having SAg activity and which have the capacity to block SAg activity.

34. Cell-line transfected with and expressing a human retrovirus or a portion thereof or a nucleic acid molecule according to any one of claims 11 to 25.

35. Non-human cells transformed with and expressing a human retrovirus or a nucleic acid molecule according to any one of claims 11 to 25.

36. Cell-line or cells according to claim 34 or 35, said cell-lines or cells being MHC Class II⁺ and expressing a protein having SAg activity.

37. Process for identifying substances capable of binding to retroviral protein or peptide according to any one of claims 26 to 30, comprising contacting the substance under test, optionally labelled with detectable marker, with the said retroviral protein or peptide having SAg activity, and detecting binding.

38. Process for identifying substances capable of blocking SAg activity of an endogenous retrovirus associated with autoimmune disease, comprising introducing the substance under test into an assay system comprising i) MHC Class II⁺ cells functionally expressing retroviral protein or peptide according to any one of claims 26 to 30 and ii) cells bearing V β -T cell receptor chains of the family or families specifically stimulated by the HERV SAg expressed by the MHC Class II⁺ cells, and determining the capacity

of the substance under test to diminish or block V β -specific stimulation by the retroviral SAg.

39. Process according to claim 38 wherein the cells bearing V β -T cell receptor chains are T-cell hybridoma and V β -specific stimulation is determined for example by measurement of IL-2 release, or measurement of T-cell proliferation.

40. Process according to claim 38 or 39, comprising an additional preliminary screening step for selecting substances capable of binding to retroviral protein having SAg activity, said screening step being according to claim 38.

41. Process for identifying substances capable of blocking transcription or translation of human endogenous retroviral (HERV) SAg-encoding nucleic acid sequences, said SAg being associated with a human autoimmune disease, comprising :

i) contacting the substance under test with cells expressing endogenous retroviral protein or peptide having SAg activity, according to any one of claims 26 to 30 and

ii) detecting loss of SAg protein expression using SAg protein markers such as specific, labelled anti-SAg antibodies.

42. Process according to claim 41 the cells expressing HERV protein having SAg activity are MHC

Class II⁺ cells, and the process further comprises detection of loss of SAg activity by the process of claim 38.

43. Kit for screening substances capable of blocking SAg activity of a retrovirus associated with an autoimmune disease, or of blocking transcription or translation of the retroviral SAg protein, comprising :

- MHC Class II⁺ cells transformed with and functionally expressing said retroviral SAg ;
- cells bearing V β T-cell receptor chains of the family or families specifically stimulated by the HERV SAg ;
- means to detect specific V β stimulation by HERV SAg ;
- optionally, labelled antibodies specifically binding to the retroviral SAg.

44. Protein or peptide derived from a retroviral SAg according to claim 26 wherein the protein is modified so as to be devoid of SAg activity and is capable of generating a immune response against SAg, involving either antibodies and/or T-cell responses.

45. Protein according to claim 44 wherein the modification consists of denaturation, or of a truncation, or of a deletion, insertion or replacement mutation of the SAg protein.

46. Protein according to claim 44 or 45 for use as a prophylactic or therapeutic vaccine against autoimmune disease associated with retroviral SAg.

47. Vaccine comprising an immunogenically effective amount of a protein according to claim 44 or 45 in association with a pharmaceutically acceptable carrier and optionally adjuvant.

48. Nucleic acid molecule encoding human retroviral SAg according to claim 15 or a modified form of said molecule for use as a prophylactic or therapeutic DNA vaccine against autoimmune disease associated with the retroviral SAg.

49. Substances identifiable by the process according to any one of claims 37 to 42 for use in therapy and/or prevention of autoimmune disease associated with the HERV SAg.

50. Use of substances capable of inhibiting retroviral function for the preparation of a medicament for use in therapy and/or prevention of autoimmune disease associated with retroviral SAg.

51. Use according to claim 50 wherein the substance capable of inhibiting retroviral function is Azido Deoxythymidine (A.Z.T.).

52. Use of substances capable of inhibiting retroviral SAg function for the preparation of a medicament for use in therapy of autoimmune disease associated with retroviral SAg.

53. Process for detecting human autoimmune disease associated with expression of human endogenous retrovirus Superantigen (SAg), said process comprising at least one of the following steps :

- i) detecting the presence of any expressed retrovirus in a biological sample of human origin ;
- ii) detecting the presence of SAg activity in a biological sample of human origin containing MHC Class II⁺ cells.

54. Process according to claim 53 wherein the expressed retrovirus is detected by detection of reverse transcriptase activity.

55. Process according to claim 54 wherein the expressed retrovirus is detected by carrying out nucleic acid amplification reaction on RNA prepared from the biological sample, using as 3' primer a sequence complementary to known retroviral « primer binding sites » (pbs), and as 5' primer a non-specific anchor sequence.

56. Process according to claim 53 wherein the presence of SAg activity is detected by contacting the biological sample containing MHC Class II⁺ cells with cells bearing one or more variable (V)- β T-cell receptor (TCR) chains and detecting preferential proliferation of a V β subset.

57. Process according to claim 56 wherein the cells bearing T-cell receptors are T-cell hybridoma bearing defined human V β domains.

58. Process for detecting SAg activity of an expressed human retrovirus associated with human autoimmune disease or of a portion of said retrovirus comprising :

i) transfecting expressed retroviral DNA or portions thereof into MHC Class II⁺ antigen presenting cells under conditions in which the DNA is expressed,

ii) contacting the transfectants with cells bearing one or more defined (V)- β T-cell receptor chains, and

iii) determining whether the transfectant is capable of inducing preferential proliferation of a V β subset, the capacity to induce preferential proliferation being indicative of SAg activity within the transfected DNA or portion thereof.

59. Process for isolating and characterising a human retrovirus, particularly a human endogenous retrovirus (HERV), said retrovirus having SAg activity and being involved in human autoimmune disease, comprising the following steps :

i) isolation of the 5' R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3' primer being complementary to known « primer binding sites » (pbs) ;

ii) isolation of the 3' R-poly(A) ends corresponding to the 5' R-U5 ends, by use of primers specific for the R regions isolated in step i) :

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region ;

iv) amplification of the 5' moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3' end of the central pol region isolated in step iii) ;

v) amplification of the 3' moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3' R-poly(A) sequences isolated in step ii) ;

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

60. Process according to claim 59 further comprising a step vii) of detecting SAg activity associated with the retrovirus, or portions thereof, said detection being carried out according to claim 58.

61. Transgenic animal including in its genome non-human cells according to claim 35.

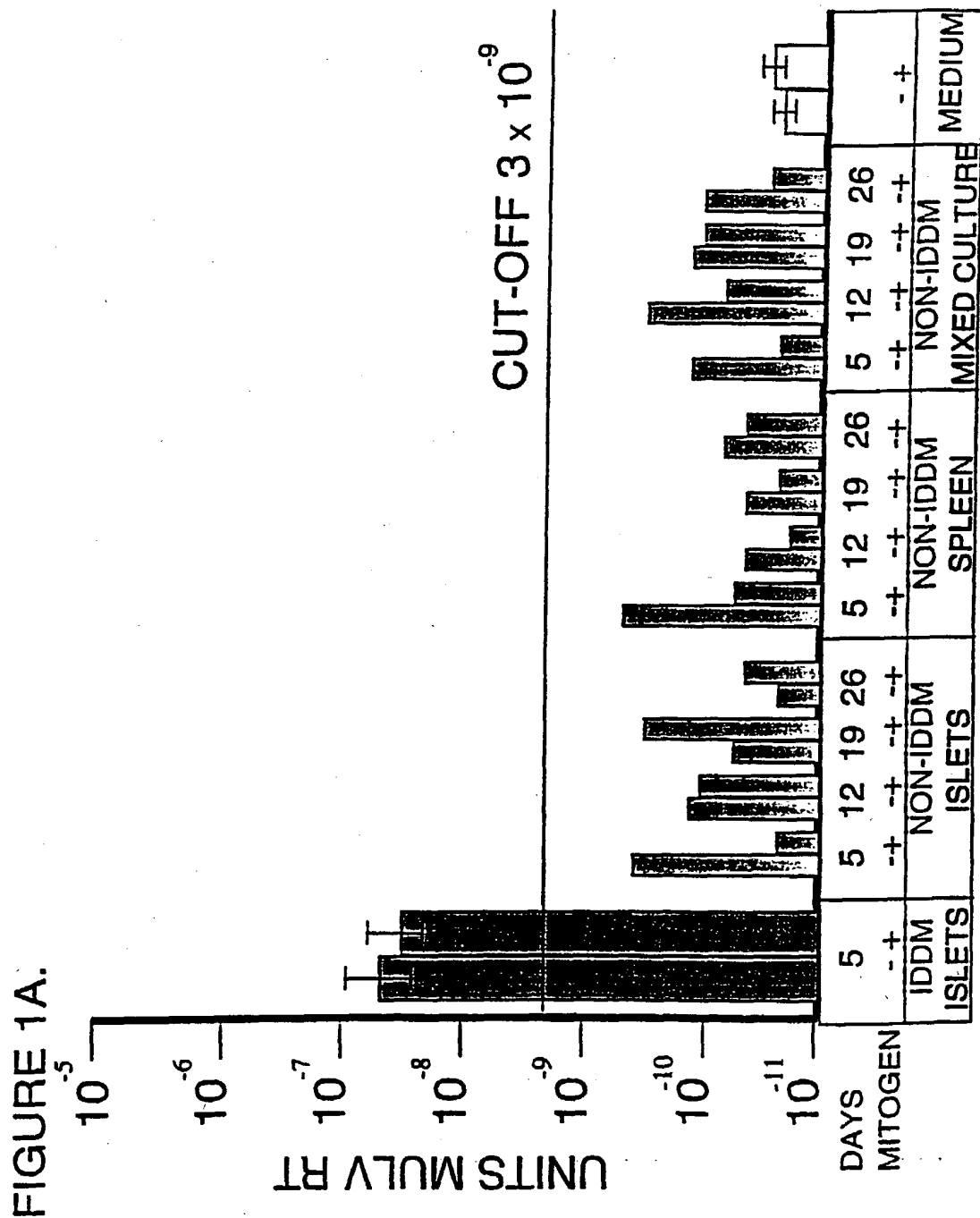


FIGURE 1B.

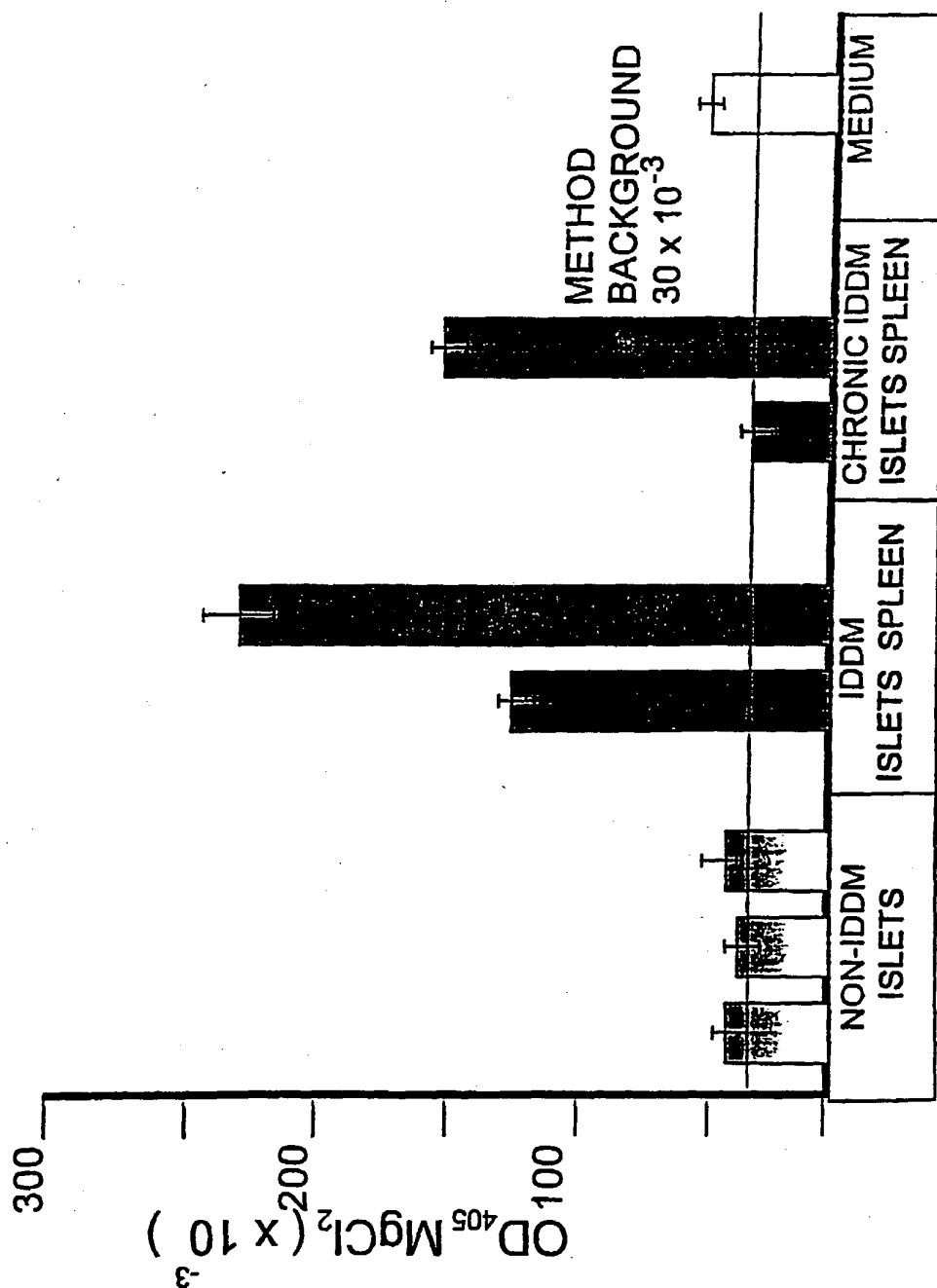


FIGURE 2A.

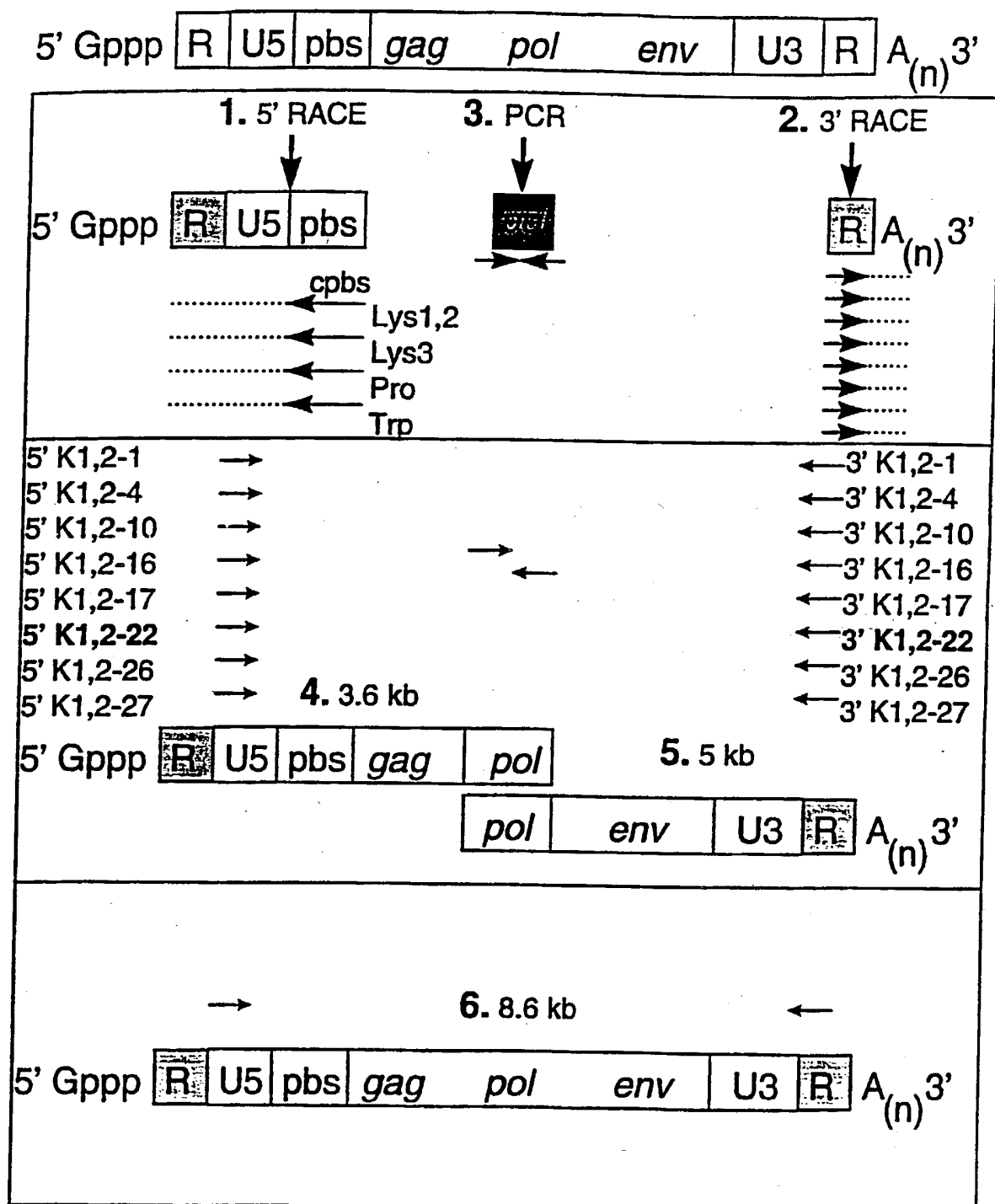


FIGURE 2B

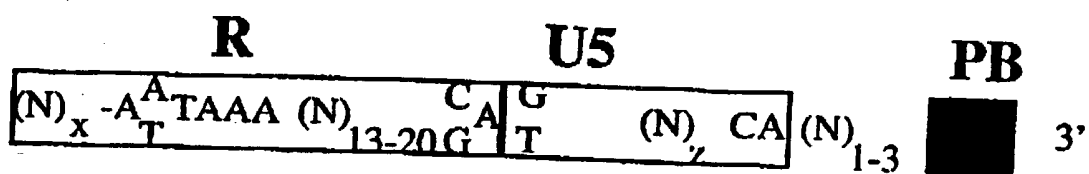


FIGURE 2C

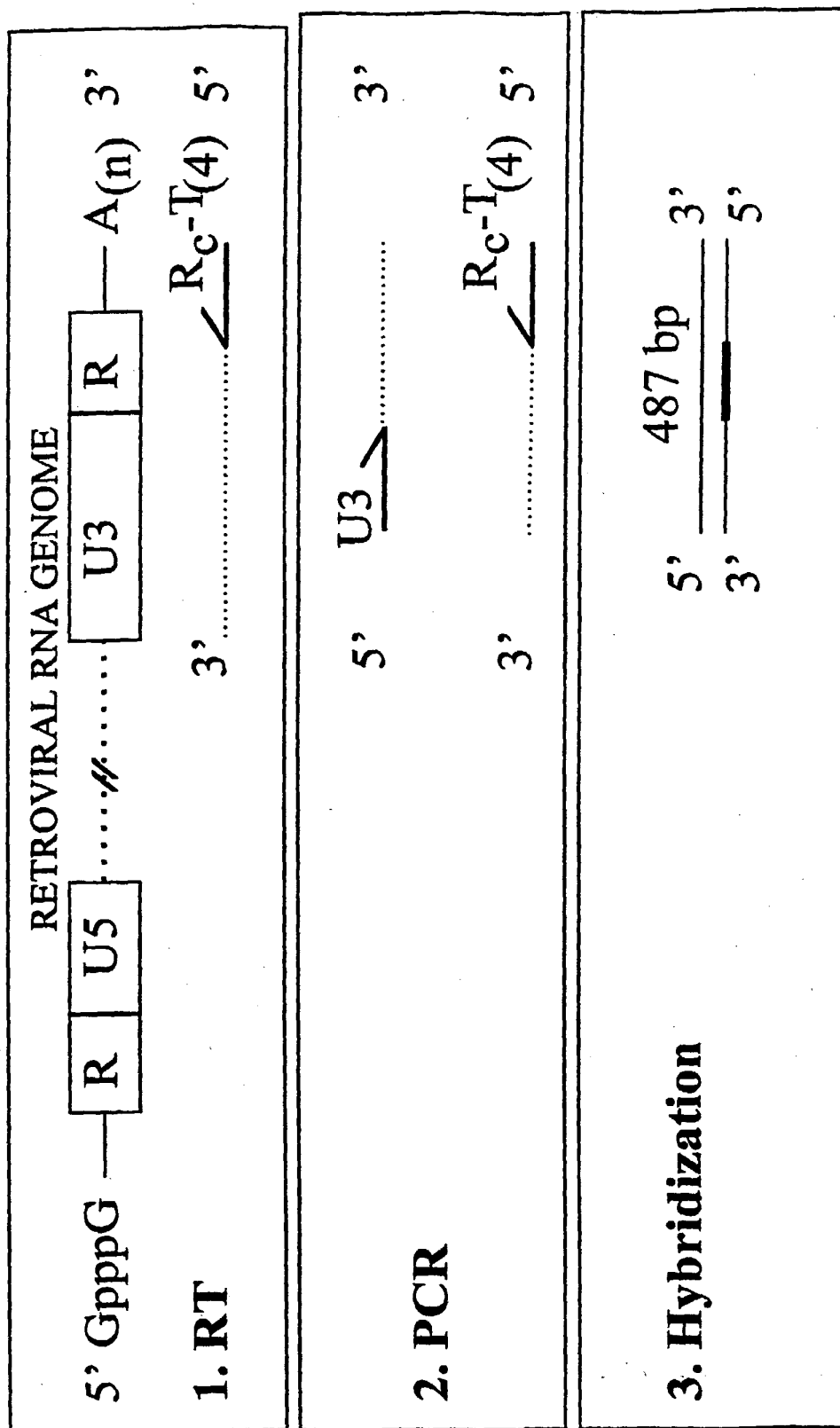


FIGURE 2D

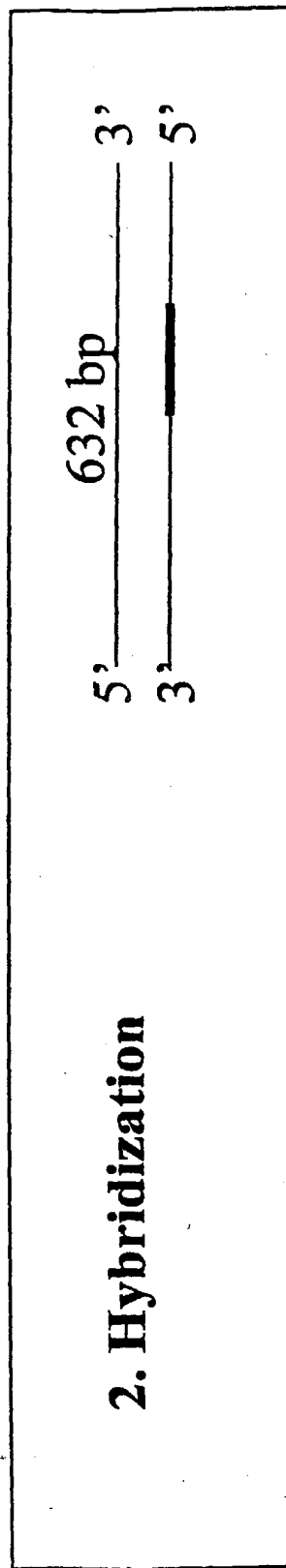
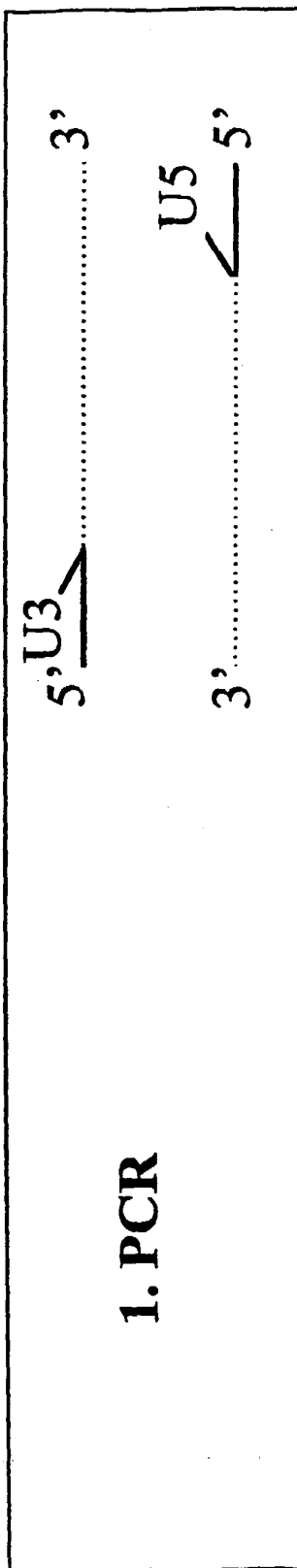
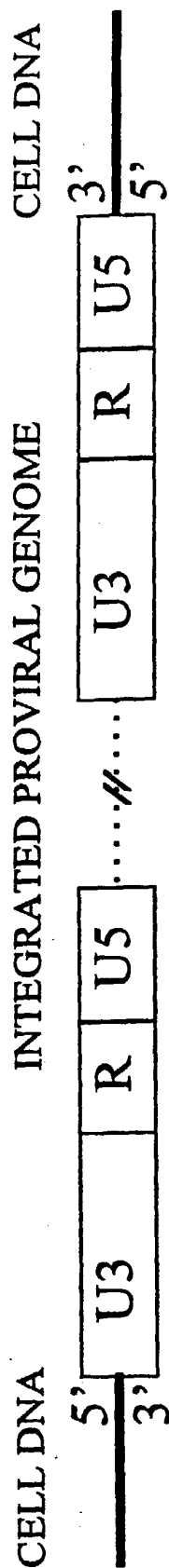


FIGURE 2E

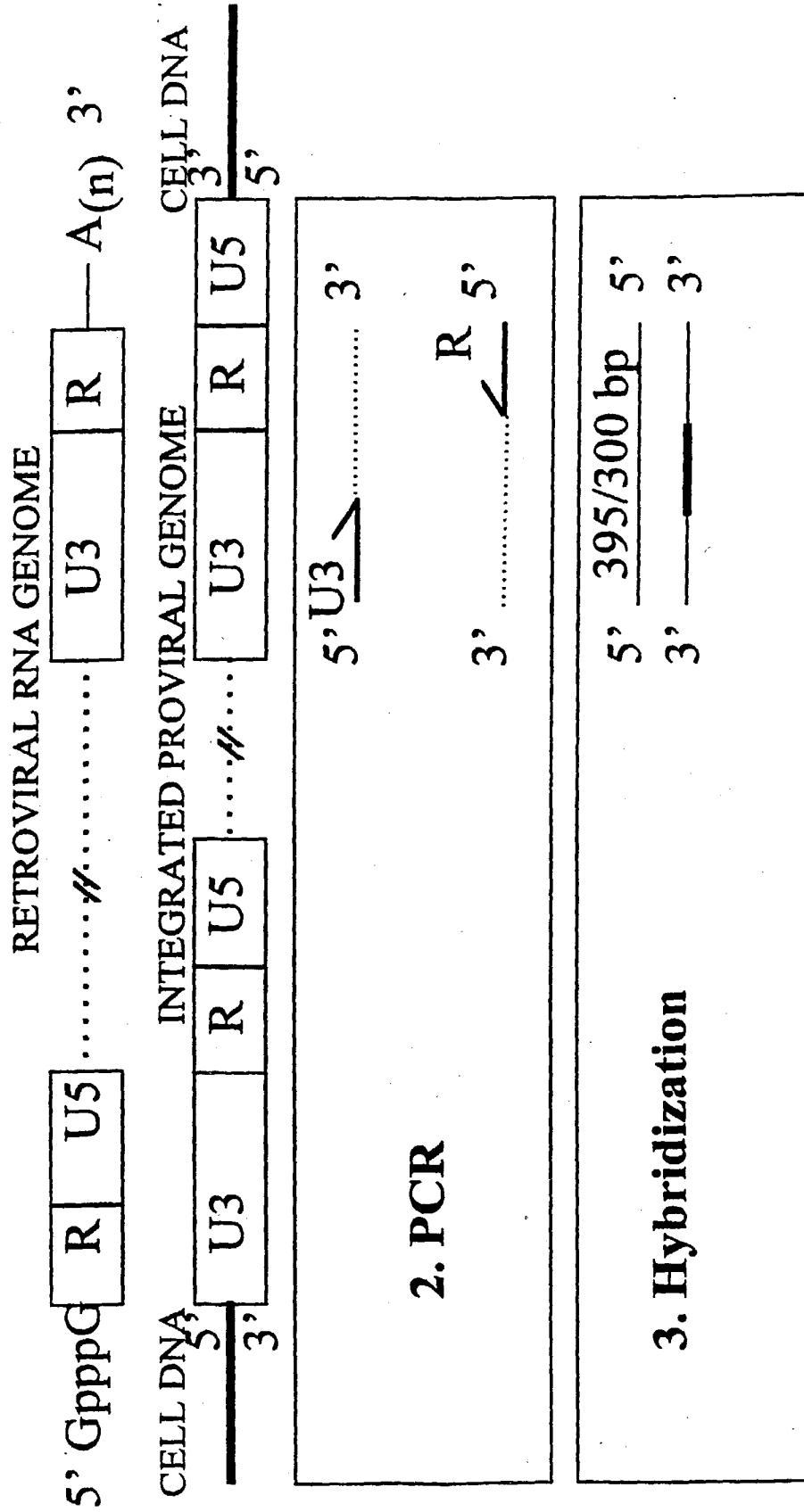


FIGURE 2F











TEMPLATE	SPECIFICITY	IDDM PATIENTS (n=10)	CONTROLS (n=10)
RNA	RT+ U3-R		
	RT+ U3-R-POLY(A)		
	RT- U3-R		
	RT- U3-R-POLY(A)		
DNA	U3-R		

FIGURE 3A.

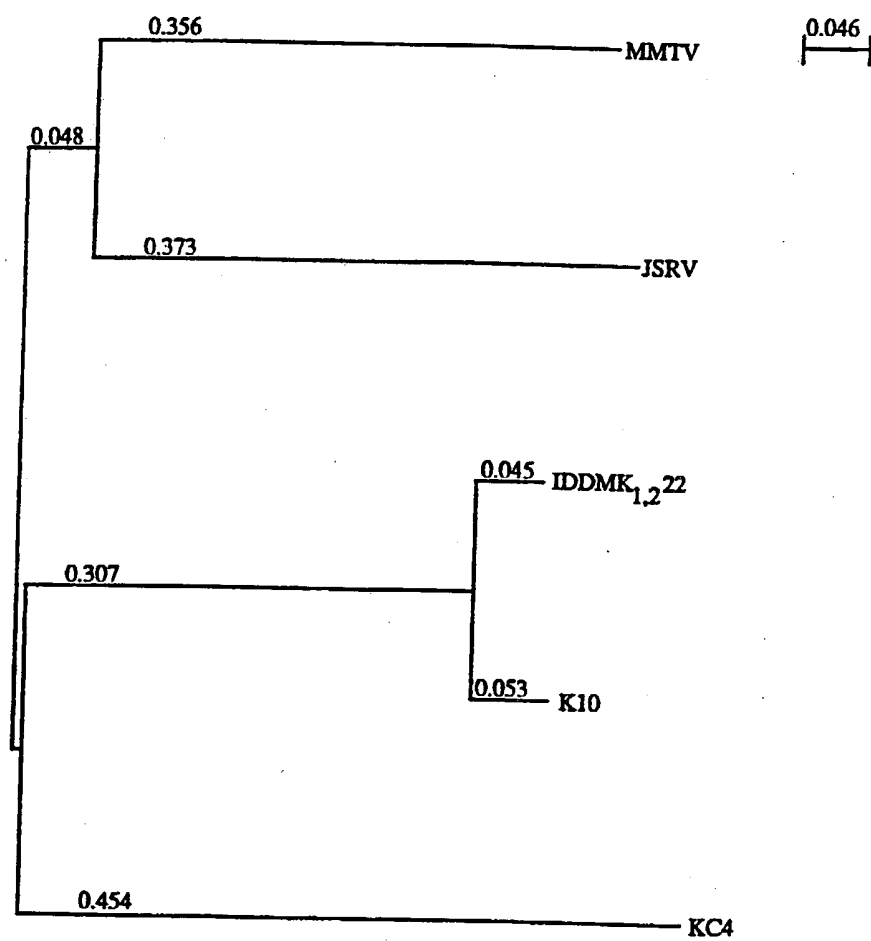


FIGURE 3B.

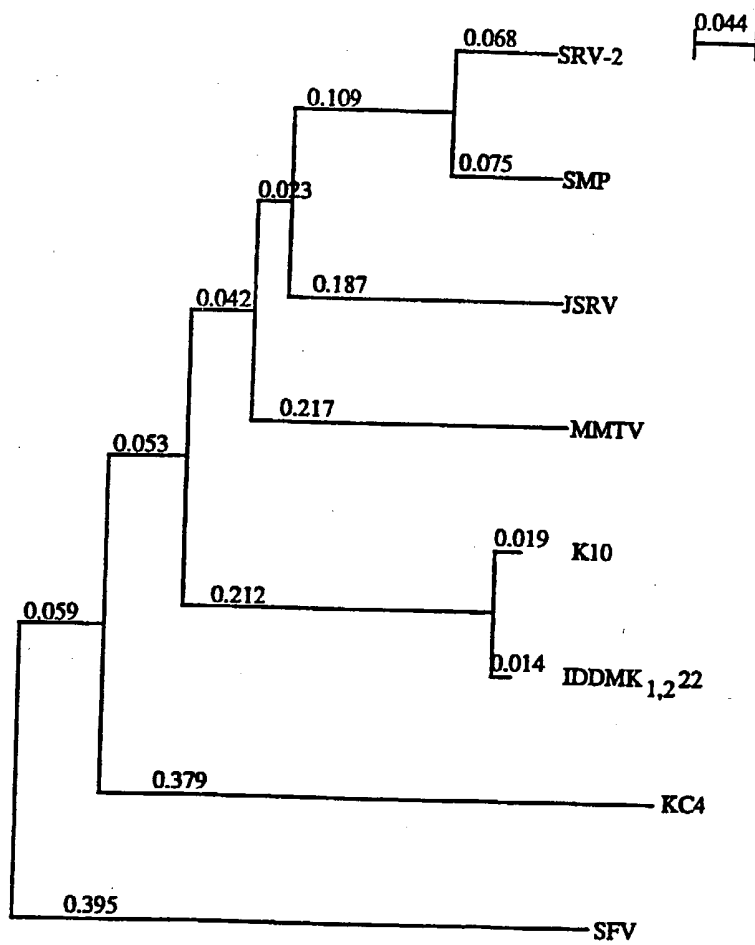


FIGURE 3C.

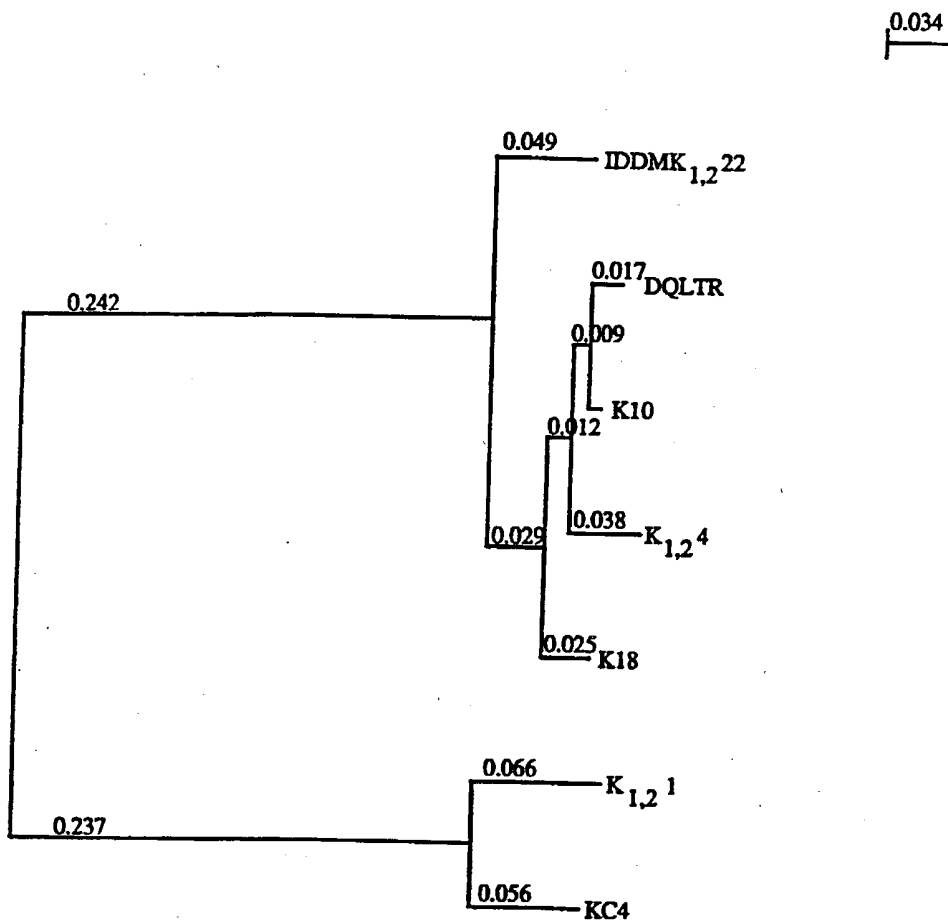


FIGURE 4A.

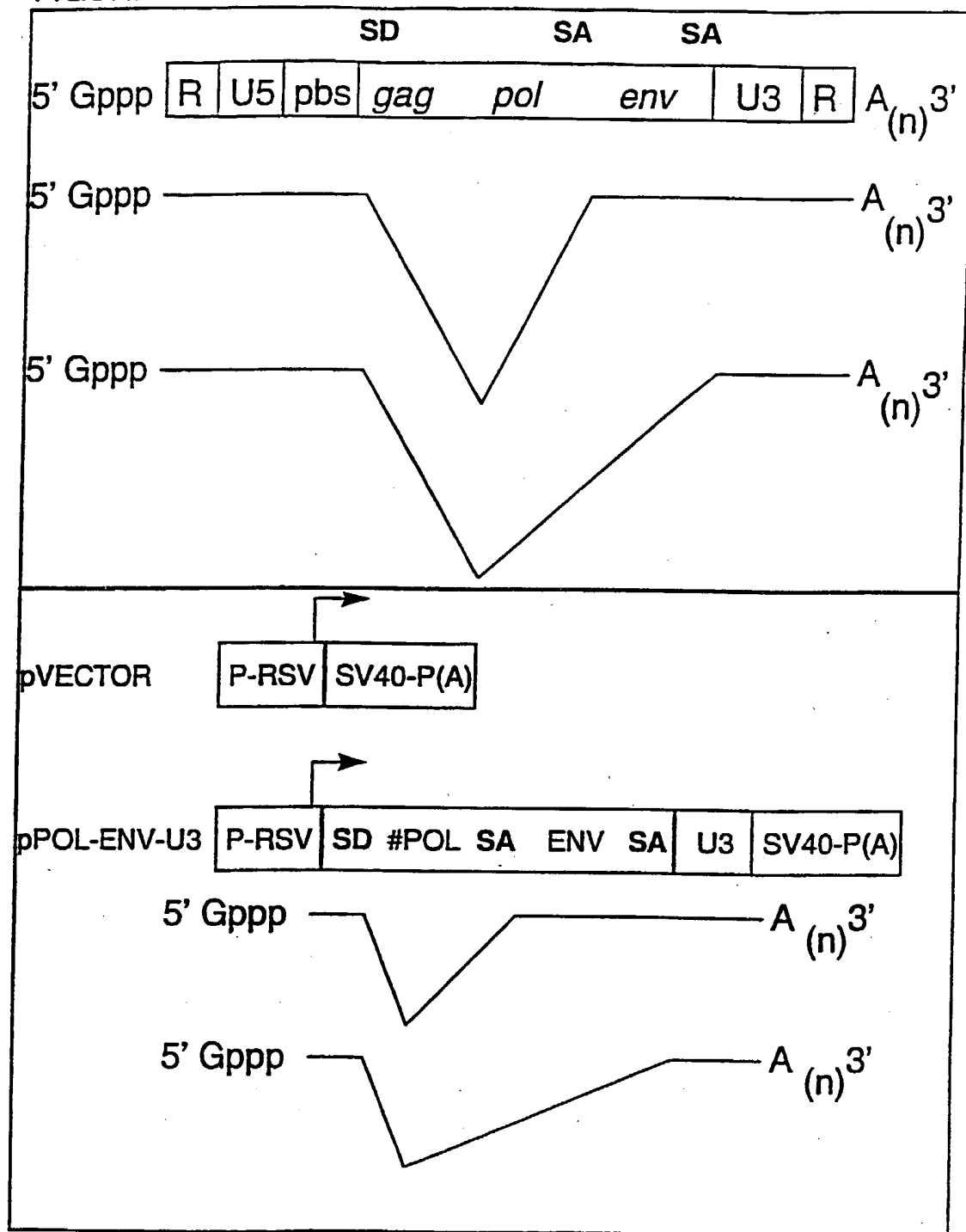


FIGURE 4B.

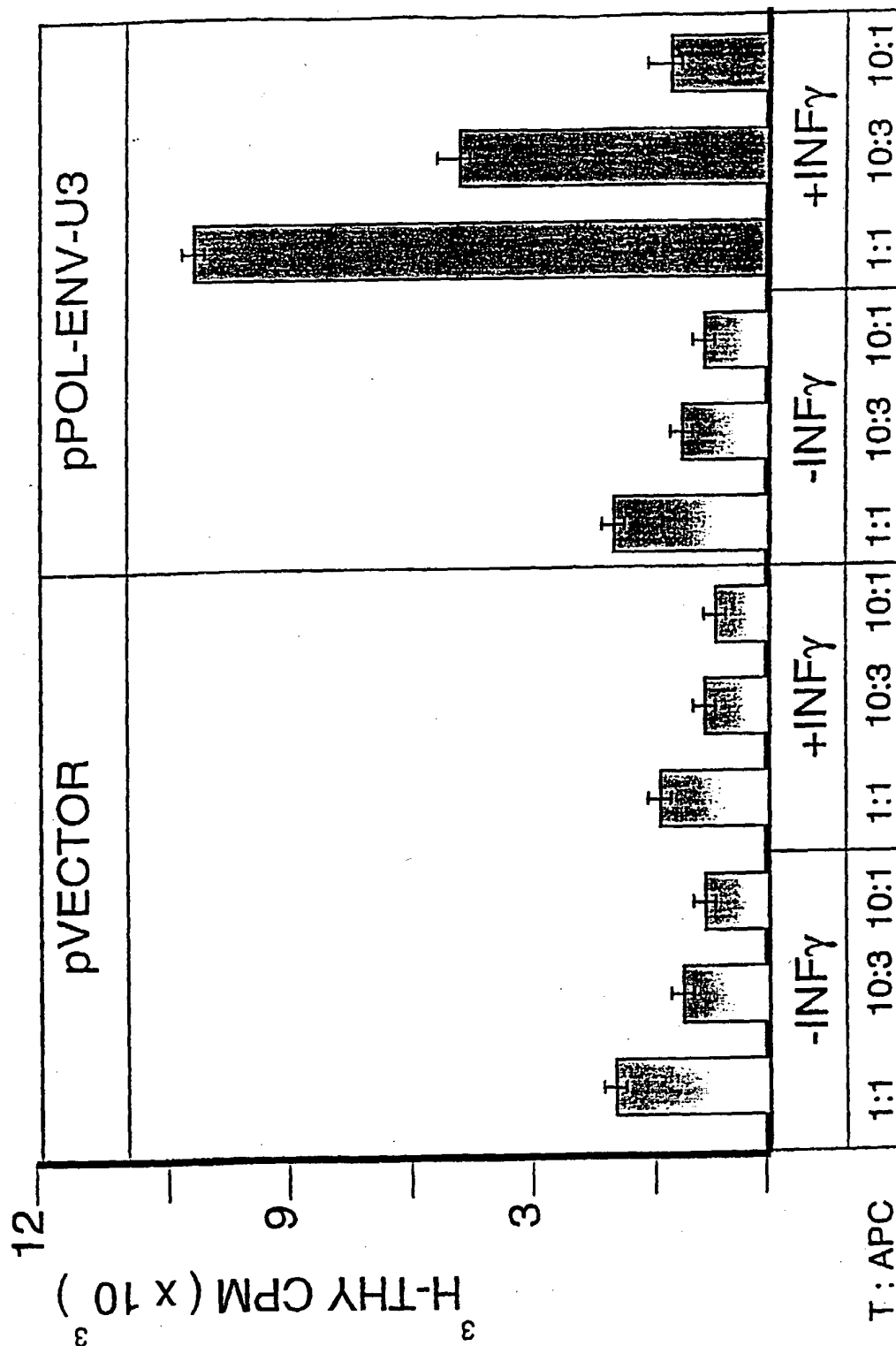


FIGURE 4C.

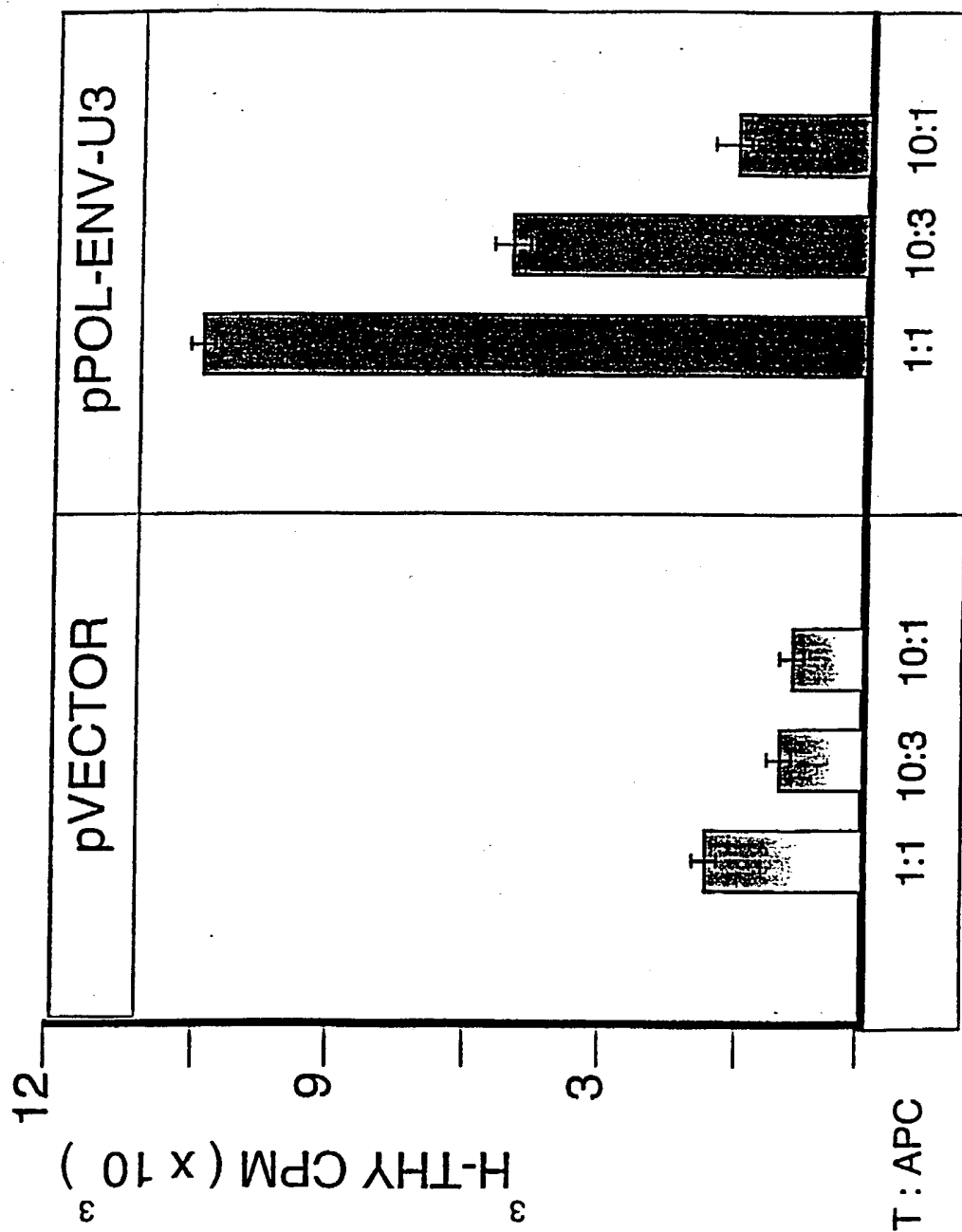


FIGURE 4D.

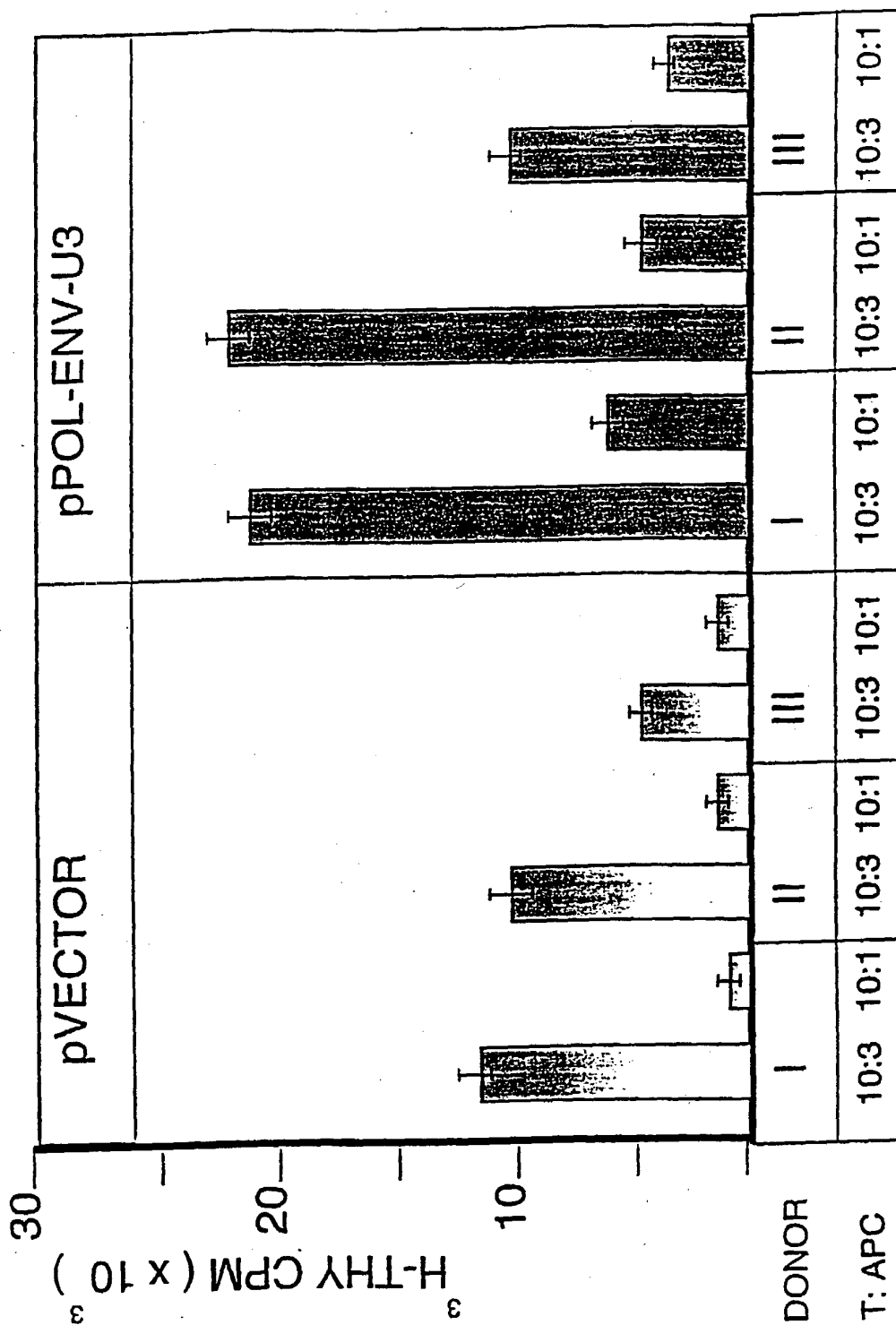


FIGURE 5.

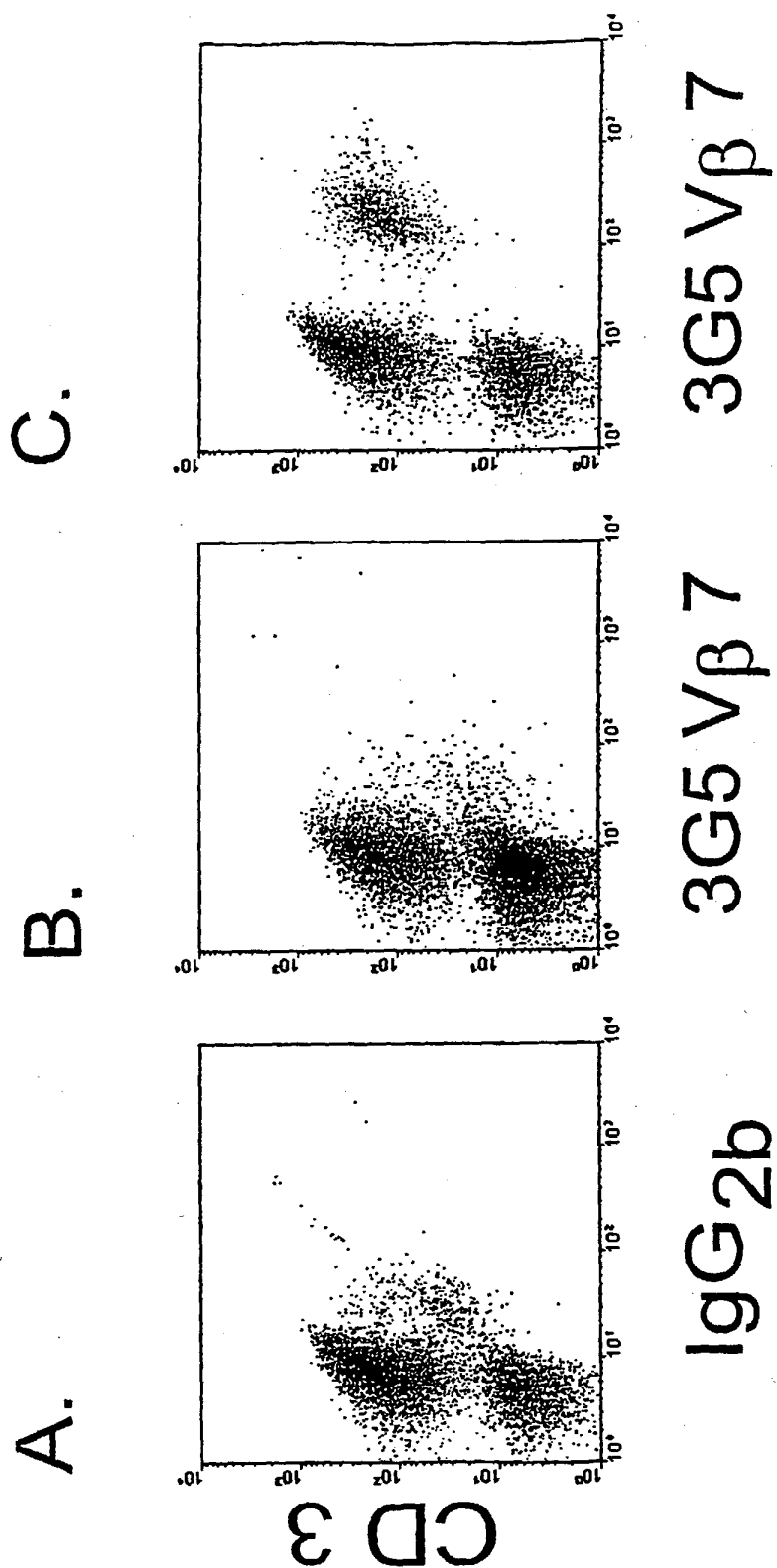
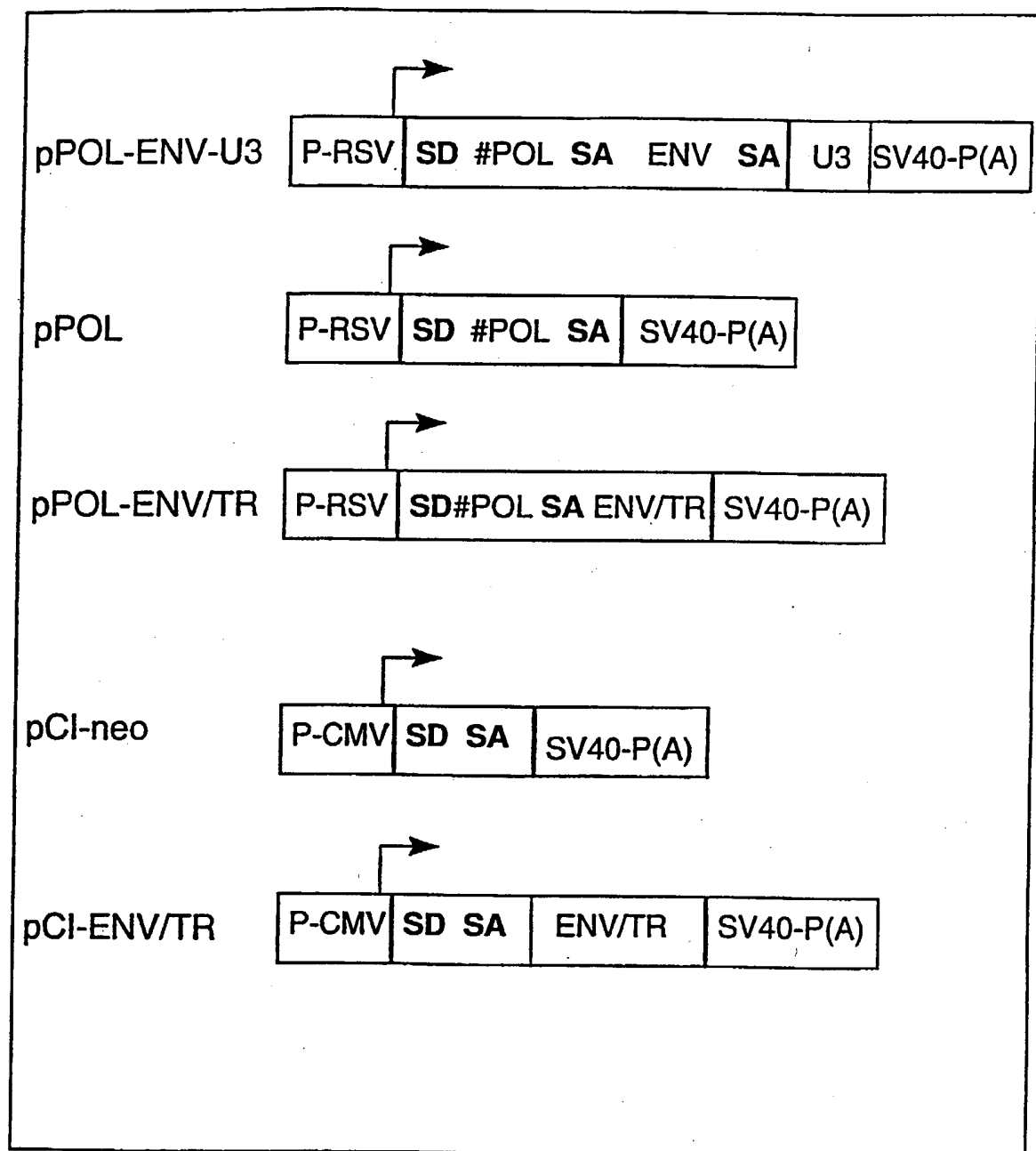


FIGURE 6A.



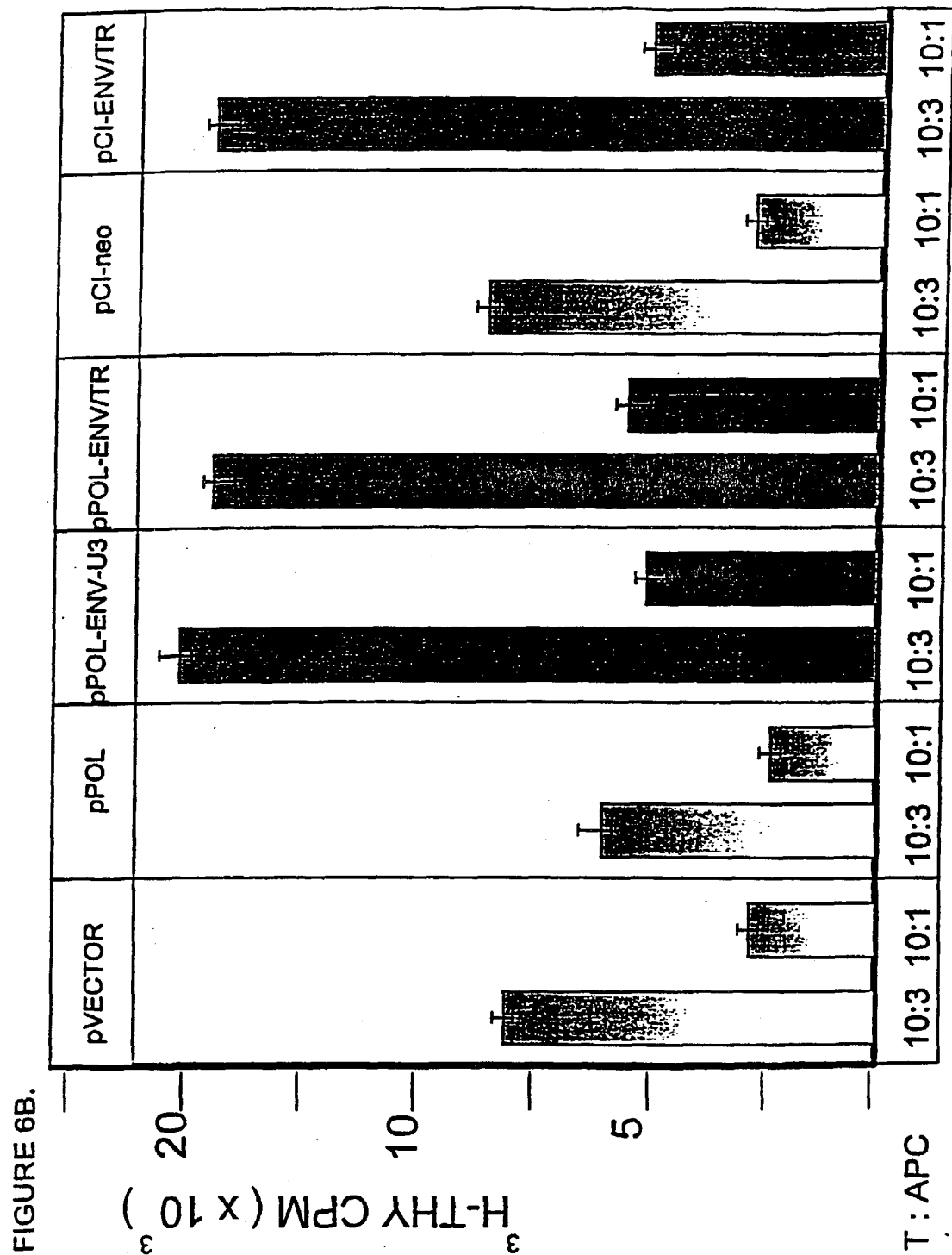


FIGURE 7A

iddmk1,2 22-5'1tr

CATCTCCCTCAGGAGAAACACCCACGAATGATCAATAAATACTAAGGGGACTCAGAGGCTGGT
GGGATCCTCCATATGCTGAACGTTGGTCCCCGGGGCCCCCTTATTTCTTTCTCTATACTTTGT
CTCTGTGTCTTTTTCTTTTCCAAGTCTTCTTCATTTGCACCTTACGAGAAACATCTCCATCAT
GGTTGTTGGATGGGGGCAA

FIGURE 7B

iddmk1,2 22-3'1tr

CTGCAGGTGTACCCAACAGCTCCGAAGAGACAGTGAACATCGAGAACGGGCCATGATGACGATG
GCGGTTTTGTGCGAAAAGAAAAGGGGGAAATGTGGGGAAAAGCAAGAGAGATGAGATTGTTACT
GTGTCTGTATAGAAAAGAAGTAGACATAGGAGACTCCATTTTGTCTGTACTAAGAAAAATTCT
TCTGCCCTTGAGATGCTGTTAATCTATGACCTTACCCCCAACCCCGTGCTCTCTGAAACATGTG
CCGTGTCAAACTCAGGGTTAAATGGATTAAGGGTGGTGCAAGATGTGCTTTGTTAAACAGATG
CTTGAAGGCAGCATGCTCATTAAAGAGTCATCACCCTCCCTAATCTCAAGTACCCAGGGACAC
AAACACTGCGAAAGGCCGAGGGACCTCTGCCTAGGAAAGCCAGGTATTGTCCAAGGTTTCTC
CCCATGTGATAGTCTGAAATATGGCCTCGTGGAAGGGAAAGACCTGACCATCCCCCAGACCA
ACACCCGTAAAGGGTCTGTGCTGAGGAGGATTAGTATAAGAGGAAAGCATGCCTCTTGCAGTT
GAGAGAAGAGGAAGACATCTGTCTCCTGCCCATCCCCGTTGGGCAATGGAATGTCTCAGTATAAA
ACCCGATTGAACATTCCATCTACTGAGATAGGGAAAAACTGCCTTAGGGCTGGAGGTGGGACA
TGTGGGCAGCAATACTGCTTTGTAAAGCATTGAGATGTTTATGTGTATGTATATCTAAAAGCA
CAGCACTTGATCCTTTACCTTGTCTATGATGCAACACCTTTGTTACGTGTTTGTCTGCTGA
CCCTCTCCCCACTATTGTCTTGTGACCCTGACACATCTCCCTCAGGAGAAACACCCacgaatg
atcaataaataactaaggggactcagaggctggtgggatcctccatatgctgaacggttggttcc
cggggcccccttatttctttctctataactttgtctctgtgtctttttcttttccaagtcttct
tcatttgcaccttacgagaacatctccatcatggttggatgggggcaa

FIGURE 7C

iddmk1,2 22-env

ATGGTAACACCAAGTCACATGGATGGATAATCCTATAGAAGTATATGTTAATGATAGTGTATGG
GTACCTGGCCCCACAGATGATCGCTGCCCTGCCAAACCTGAGGAAGAAGGGATGATGATAAAT
ATTTCCATTGGGTATCATTATCCTCCTATTTGCCTAGGGAGAGCACCAGGATGTTTAATGCCT
GCAGTCCAAAATTGGTTGGTAGAAGTACCTACTGTCACTCCTAACAGTAGATTCACTTATCAC
ATGGTAAGCGGGATGTCACTCAGGCCACGGGTAAATTATTTACAAGACTTTTCTTATCAAAGA
TCATTAAAATTTAGACCTAAAGGGAAAACTTGCCCCAAGGAAATTCCTAAAGGATCAAAGAAT
ACAGAAGTTTTAGTTTGGGAAGAATGTGTGGCCAATAGTGTGGTGATATTACAAAACAATGAA
TTCGGAACCTATTATAGATTAGGCACCTCGAGGTCAATTCTACCACAATTGCTCAGGACAAACT
CAGTCGTGTCCAAGTGACAAGTGAGTCCAGCTGTGATAGCGACTTAACAGAAAGTCTAGAC
AAACATAAGCATAAAAAATTACAGTCTTTCTACCTTTGGGAATGGGAAGAAAAAGGAATCTCT
ACCCCAAGACCAAAAAATAATAAGTCCTGTTTCTGGTCTGAAATCCAGAATTGTGGAGGCTT
ACTGTGGCCTCACACCACATTAGAAATTTGGTCTGGAAATCAAACCTTTAGAAACAAGATATCGT
AAGCCATTTTATACTATCGACCTAAATTCATTCTAACGGTTCCTTTACAAAGTTGCCTAAAG
CCCCCTTATATGCTAGTTGTAGGAAATATAGTTATTAACCAGCCTCCCAAATATAACCTGT
GAAAATTGTAGATTGTTTACTTGCATTGATTCAACTTTTAATTGGCAGCACCGTATTCTGCTG
GTGAGAGCAAAGAGAAGGCATGTGGATCCCTGTGTCCACGGACCGACCGTGGGAGGCCTCGCCA
TCCATCCATATTTTGAAGTAAATATTAAGGGCGTTTTAAATAGATCCAAAAGATTCAATTTT
ACTTTAATTGCAGTGATTATGGGATTAATTGCAGTCACAGCTACGGCTGCTGTGGCAGGGGTT
GCATTGCACTCTTCTGTTCACTCAGTAAACTTTGTTAATTATTGGCAAAGAATTCTACAAGA
TTGTGGAATTCACAATCTAGTATTGATCAAAAATTGGCAAGTCAAATTAATGATCTTAGACAA
ACTGTCAATTTGGATGGGAGACAGGCTTGACTTAGAACATCATTTCCAGTTACAGTGTGACTGG
AATACGTCAAGTTTTTGTATTACACCCCAAATTTATAATGAGTCTGAGCATCACTGGGACATG
GTTAGACGCCATCTACAGGGAAGAGAAGATAATCTCACTTTAGACATTTCCAAATTAAGAA
CAAATTTTCGAAGCATCAAAGCCCATTAAATTTGGTGCCAGGAACTGAGGCAATTGCAGGA
GTTGCTGATGGCCTCGCAAATCTTAACCTGTCACTGGATTAAGACCATCAGAAGTACTATG
ATTATAAATCTCATATTAATCGTTGTGTGCCTGTTTTGTCTGTTGTTAGTCTGCAGGTGTACC
CCAACAGCTCCGAAAAAAACAGTGACATCGAGAACGGGCCATGAATGACAAAGGCGGTTTTTG
TTCCAAAAAAAAGGGGGAAATTTTGGGGAAACCAAAAAAATGAAAATGTT

FIGURE 7D

ACA TTT GAA GTT CTA CAA TGA ACC CAT CAG AGA TGC AAA GAA AAG CGC CTC CAC GGA 57

GAT GGT AAC ACC AGT CAC ATG GAT GGA TAA TCC TAT AGA AGT ATA TGT TAA TGA TAG 114
M V T P V T W M D N P I E V Y V N D S 19

TGT ATG GGT ACC TGG CCC CAC AGA TGA TCG CTG CCC TGC CAA ACC TGA GGA AGA AGG 171
Y W V P G P T D D R C P A K P E E E G 38

GAT GAT GAT AAA TAT TTC CAT TGG GTA TCA TTA TCC TCC TAT TTG CCT AGG GAG AGC 228
M M I N I S I G Y H Y P P I C L G R A 57

ACC AGG ATG TTT AAT GCC TGC AGT CCA AAA TTG GTT GGT AGA AGT ACC TAC TGT CAG 285
P G C L M P A V Q N W L V E V P T V S 76

TCC TAA CAG TAG ATT CAC TTA TCA CAT GGT AAG CGG GAT GTC ACT CAG GCC ACG GGT 342
P N S R F T Y H M V S G M S L R P R V 95

AAA TTA TTT ACA AGA CTT TTC TTA TCA AAG ATC ATT AAA ATT TAG ACC TAA AGG GAA 399
N Y L Q D F S Y Q R S L K F R P K G K 114

AAC TTG CCC CAA GGA AAT TCC TAA AGG ATC AAA GAA TAC AGA AGT TTT AGT TTG GGA 456
T C P K E I P K G S K N T E V L V W E 133

AGA ATG TGT GGC CAA TAG TGT GGT GAT ATT ACA AAA CAA TGA ATT CGG AAC TAT TAT 513
E C V A N S V V I L Q N N E F G T I I 152

AGA TTA G 520
D * 153

FIGURE 7E

k1,2-22-env/fs

ACATTTGAAGTTCTACAATGAACCCATCAGAGATGCAAAGAAAAGCGCCTCCACGGAGATGGTA
ACACCAGTCACATGGATGGATAATCCTATAGAAGTATATGTTAATGATAGTGTATGGGTACCTG
GCCCCACAGATGATCGCTGCCCTGCCAAACCTGAGGAAGAAGGGATGATGATAAAATATTTCCAT
TGGGTATCATTATCCTCCTATTTGCCTAGGGAGAGCACCAGGATGTTTAATGCCTGCAGTCCAA
AATTGGTTGGTAGAAGTACCTACTGTCAGTCCTAACAGTAGATTCACTTATCACATGGTAAGCG
GGATGTCACTCAGGCCACGGGTAAATTATTTACAAGACTTTTCTTATCAAAGATCATTAAAATT
TAGACCTAAAGGGAAAACCTTGCCCCAAGGAAATTCCTAAAGGATCAAAGAATACAGAAGTTTAA
GTTTGGGAAGAATGTGTGGCCAATAGTGTGGTGATATTACAAAACAATGAATTCGGAACATTATA
TAGATTAGGCACCTCGAGGTCAATTCTACCACAATTGCTCAGGACAAACTCAGTCGTGTCCAAG
TGCACAAGTGAGTCCAGCTGTCGATAG

FIGURE 7F

iddmk1,2 22-ENV

MVTPVTWMDNPIEVYVND SVWVPGPTDDRCPAKPEEEGMMINISIGYHYPPICLGRA
PGCLMPAVQNWLV E VPTVSPNSRFTYH MVSGMSLRPRVNYLQDFSYQRSLKFRPKG
KTCPK EIPKGSKNTEVLWEECVANSVVILQNN EFGTIDZAPRGQFYHNCSGQTQSC
PSAQVSPA VDSDLTESLDKHKHKKLQSFYLWEWEEKGISTPRPKIISPVS GPEHP EL
WRLTVASHHIRIWSGNQ TLETRYRKPFYTIDLNSILTVPLQ SCLKPPYMLVVGNIVIKP
ASQTITCENCRLFTCIDSTFNWQHRILLVRAREGMWIPVSTDRPWEASPSIHILTEILK
GVLNRSKR FIFTLIAVIMGLIAVTATAAVAGVALHSSVQSVNFVNYWQKNSTRLWNS
QSSIDQKLASQINDLRQT VIWMGDRLDLEHHFQLQCDWNTSDFCITPQIYNESEHH
WDMVRRHLQGREDNLTLDISKLKEQIFEASKAHLNLVPGTEA IAGVADGLANLNPVT
WIKTIRSTMIINLILIVVCLFCLLLVCRC TPTAPKKT VTSRTGHE

FIGURE 7C

ACATTTGAAGTTCTACAATGAACCCATCAGAGATGCAAAGAAAAGCGCCTCCACGGAGATGGT 63
AACACCAAGTCACATGGATGGATAATCCTATAGAAGTATATGTTAATGATAGTGTATGGGTACC 2
M V 126
T P V T W M D N P I E V Y V N D S V W V P 23
TGGCCCCACAGATGATCGCTGCCCTGCCAAACCTGAGGAAGAAGGGATGATGATAAATATTTT 189
G P T D D R C P A K P E E E G M M I N I S 44
CATTGGGTATCATTATCCTCCTATTTGCCCTAGGGAGAGCACCAGGATGTTTAAATGCCTGCAGT 252
I G Y H Y P P I C L G R A P G C L M P A V 65
CCAAAATTGGTTGGTAGAAGTACCTACTGTCTAGTCTTAACAGTAGATTCACTTATCACATGGT 315
Q N W L V E V P T V S P N S R F T Y H M V 86
AAGCGGGATGTCACTCAGGCCACGGGTAAATTATTTACAAGACTTTTCTTATCAAAGATCATT 378
S G M S L R P R V N Y L Q D F S Y Q R S L 107
AAAATTTAGACCTAAAGGGAAAACCTGCCCCAAGGAAATTCCTAAAGGATCAAAGAATACAGA 431
K F R P K G K T C P K E I P K G S K N T E 128
AGTTTTAGTTTGGGAAGAATGTGTGGCCAATAGTGTGGTGATATTACAAAACAATGAATTCGG 504
V L V W E E C V A N S V V I L Q N N E F G 149
AACTATTATAGATTTAGGCACCTCGAGGTCAATTCTACCACAATTGCTCAGGACAAACTCAGT 567
T I I D L G T S R S I L P O L L R T N S V 170
CGTGTCCAAGTGCACAAGTGAGTCCAGCTGTCTGATAG 601
V S K C T S E S S C R * 181

FIGURE 7H**iddmk1,2 22-POL**

FTIPLAEQDCEKFAFTIPAINNKEPATRFQWKVLPQGMLNSPTICQTFVGRALQPVRDKFSDC
YIIHYFDDILCAAETKDKLIDCYTFLPAEVANAGLAIASDKIQTSTPFHYLGMQIENRKIKPQ
KIEIRKDTLKTLDNFQKLLGDINWIRPTLGIPTYAMSNLFSILRGDSLNSKRMLT

FIGURE 8A**k1,2-1**

gtaaatgacacctatgatgcactgccaccctttcactgtttcacccctgaacatctgctttttac
atctaagtgattgtaccaataaatagtgtggagaccagagctctgagccttttgcagcctcca
ttttgcaactggtcccctggctcccacctttatgaactcttaacctgtcttttctcattcctt
gtcaccattggactttgggtaccctacgggtggtgttgaggctgtcaccgcacattaa

FIGURE 8B**k1,2-10**

gtttagttaatctataatctatagagacaatgcttatcactggcttgctgtcaataaatatgtg
ggtaaactctctgttcaagactctcagctttgaagctgtgagacccctgatttcccactccacac
ctctatatttctgtgtgtgtgtctttaattcctccagtgttgctgggtaggggtctcctcgacg
agctgtcgtgc

FIGURE 8C**k1,2-16**

aactcagctgctgcacagtgggtcgagcctccagagctcatgccattgcagtggtcagagcctg
gccctcctcttcctgcatagaacctggattcaatctgtaaggagggaagtgcagcagcagaga
actctggccttgacagagagtcctgttcccacttcactttccttttcaccaaataaaacctg
ctttcactcatgcatcaaattgtctgtgagcctacatttttgtggccatgggacaagaacacc
atcttttagctgagctagggaaaagtcctgca

FIGURE 8D**k1,2-17**

gatgtgaccactgtgacctacctacactggagatgggtcacacttccttacccttcccctgct
gtaccaataaataacagcacagcctgacattcggagccattaccggtctttgtgacttgggtgg
tagtggtatcccctagggcccagctgtcttttcttttatctctttgtcttgggtctttatttc
tatgagtctctcgtctccgcacatggggagaaaaacccatagaccctgtagggctg

FIGURE 8E**k1,2-26**

ctcacaaaaataataaaaagcttctgttggccattcttcagatcttcatctcttgtgaggatcc
ccctgtacatgtaaaaatgtaataaaaacttgatcctttctcctcttaatctgtcttgcac
atatcattcctagaccagtcagagatgggtggaggtgagccgtacatttccta

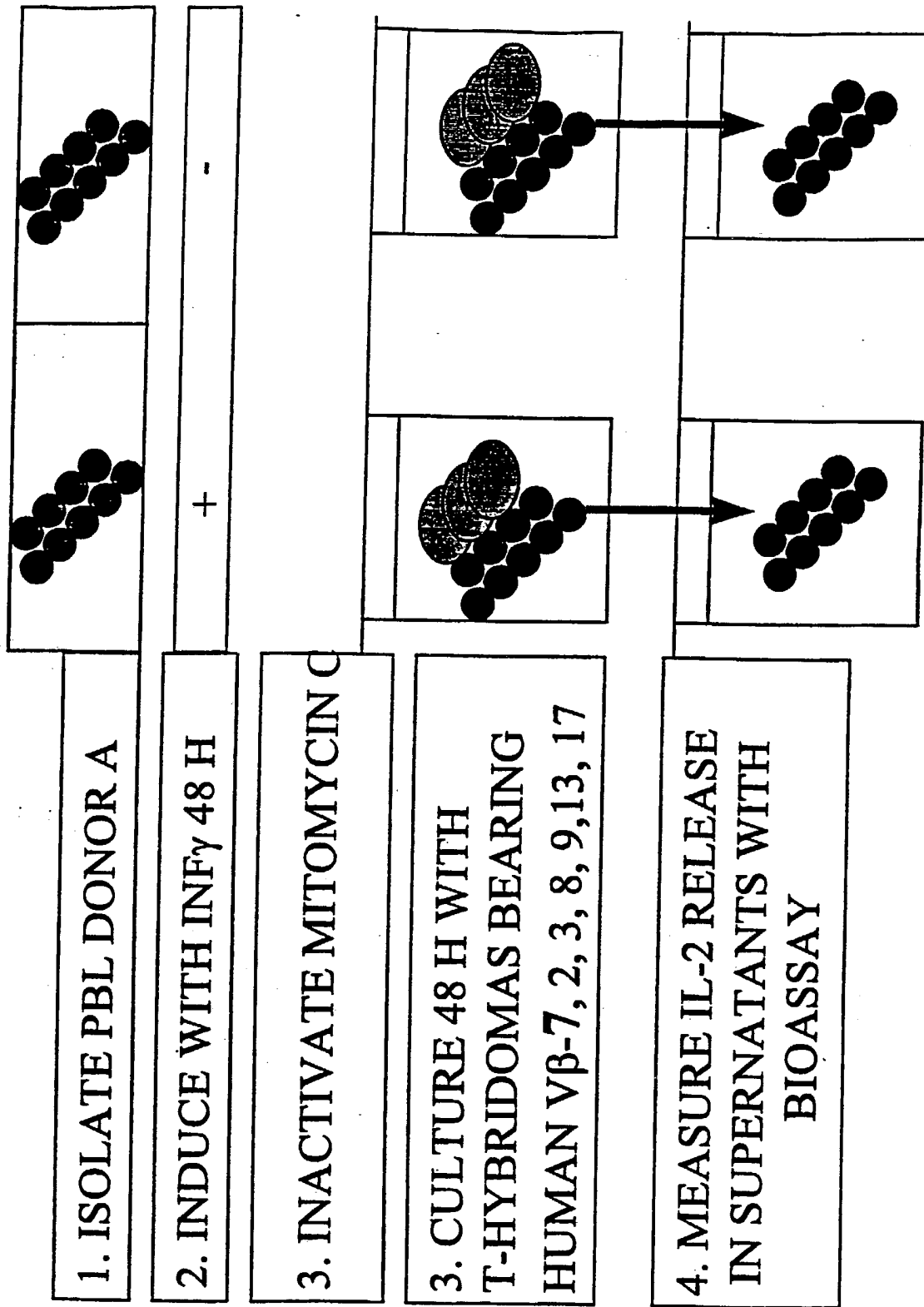
FIGURE 8F**k1,2-27**

cagagaactccagccagctgtgatggagcctcaggaagttcacagttgcagcaggaaggagcctggc
tgctcctcttcctgtgtggaacctgggattagaacaggctggcaggaagtgcttttagcagggactct
ggcctactcacactccttggtttccccctttcttccttttcaactcaataaagccctgtcttactcac
cattcaaattgtctgtgagcctgaattttcatggctgtgggacaaagaaccctatttttagctgaac
taaggaaaattcctgcaaa

FIGURE 8G**k1,2-4**

gtgattgtctgctgaccctctccccacaattgtcttgtagaccctgacacatccccctcttoga
gaaacacccgcggatgatcaataaatattaagggaactcagaggctggcaggatcctccatat
gctgaacgctggttgcgggtcccccttctttcttctatactttgtctctgtgtctttt
tcttttccaaatctctcgtcccaccttacgagaaacacccacagggtgtgtccgggcaaccaa
cgccacataaca

FIGURE 9



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: MEDIGEN SA - c/o LENZ and STAHELIN
- (B) STREET: 25 Grand Rue
- (C) CITY: GENEVA
- (E) COUNTRY: SWITZERLAND
- (F) POSTAL CODE (ZIP): CH 1211

- (ii) TITLE OF INVENTION: METHODS FOR DIAGNOSIS AND THERAPY OF
AUTOIMMUNE DISEASE, SUCH AS INSULIN DEPENDENT DIABETES
MELLITUS, INVOLVING RETROVIRAL SUPERANTIGENS

- (iii) NUMBER OF SEQUENCES: 46

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: PCT/EP

2/27

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "DNA (synthetic)"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..25
- (D) OTHER INFORMATION: /note= "page 11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTTTGAGTC CCCTTAGTAT TTATT

25

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /note= "page 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATCCAACAAC CATGATGGAG

20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Synthetic DNA"

3/27

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..21
- (D) OTHER INFORMATION:/note= "page 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCTCGTAAGG TGCAAATGAA G

21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..21
- (D) OTHER INFORMATION:/note= "PAGE 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTAAAGGATC AAGTGCTGTG C

21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTTTACAAAG CAGTATTGCT GC

22

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..23
- (D) OTHER INFORMATION:/note= "RT 1a page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

YAAATGGMGW AYGTAACAG ACT

23

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..23
- (D) OTHER INFORMATION:/note= "RT 1b page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

YAAATGGMGW AYGTAACAG ACT

23

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

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(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..28
- (D) OTHER INFORMATION:/note= "RT 2a-nested page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGTCTAGAGC CTCTCCGGCA TGATCCCG

28

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..28
- (D) OTHER INFORMATION:/note= "RT 2b-nested page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGTCTAGAGC CTCTCCGGCA TGATCCCA

28

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..21
- (D) OTHER INFORMATION:/note= "Common 5' anchor page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGCGCCAGCA ATGTATCCAT G

21

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(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "SYNTHETIC DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1..21
 - (D) OTHER INFORMATION: /note = "1K1, 2-1 page 50"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGGTGGCAGT GCATCATAGG T

21

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1..22
 - (D) OTHER INFORMATION: /note = "4K1, 2-4 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGGAGAGGGT CAGCAGCAGA CA

22

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "SYNTHETIC DNA"

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(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..22
- (D) OTHER INFORMATION:/note= "K1,2-10 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GACAGCAAGC CAGTGATAAG CA

22

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..19
- (D) OTHER INFORMATION:/note= "K1,2-16 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGAACAGGGA CTCTCTGCA

19

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..20
- (D) OTHER INFORMATION:/note= "K1,2-17 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGGAAGGGTA AGGAAGTGTG

20

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(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION:1..19
 - (D) OTHER INFORMATION:/note= "K1,2-22 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGTGTTCCTC CTGAGGGAG

19

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION:1..21
 - (D) OTHER INFORMATION:/note= "K1,2-26 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GAAGAATGGC CAACAGAAGC T

21

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"

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(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..20
- (D) OTHER INFORMATION:/note= "K1,2-27 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGGAAACAAG GAGTGTGAGT

20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..39
- (D) OTHER INFORMATION:/note= "U3-R-poly(AS) common page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CATGTATATG CGGCCGCTGC GCCAGCAATG TATCCATGG

39

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..21
- (D) OTHER INFORMATION:/note= "RT1 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TATCTTTCGT TTCTGCAGCA C

21

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(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION:1..22
 - (D) OTHER INFORMATION:/note= "RT2 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TAACTGGTTG AAGAGCTCGA CC

22

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION:1..21
 - (D) OTHER INFORMATION:/note= "R-U5-1 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ATACTAAGGG GACTCAGAGG C

21

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"

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(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION:1..27
- (D) OTHER INFORMATION:/note= "R-U5-2 page 51"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CAGAGGCTGG TGGGATCCTC CATATGC

27

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION:1..25
- (D) OTHER INFORMATION:/note= "page 52"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TTTTTGAGTC CCCTTAGTAT TTATT

25

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION:1..22
- (D) OTHER INFORMATION:/note= "Page 52"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AGGTATTGTC CAAGGTTTCT CC

22

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(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "SYNTHETIC DNA"

- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:1..22
 (D) OTHER INFORMATION:/note= "page 52"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTTTACAAAG CAGTATTGCT GC

22

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:1..21
 (D) OTHER INFORMATION:/note= "page 52"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTAAAGGATC AAGTGCTGTG C

21

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

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- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:1..29
 (D) OTHER INFORMATION:/note= "page 53"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GACTAAGCTT AAGAACCCAT CAGAGATGC

29

- (2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:1..31
 (D) OTHER INFORMATION:/note= "page 53"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

AGACTGGATC CGTTAAGTCG CTATCGACAG C

31

- (2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 208 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "RETROVIRAL DNA"

- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:1..208
 (D) OTHER INFORMATION:/note= "FIGURE 7A"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CATCTCCCTC AGGAGAAACA CCCACGAATG ATCAATAAAT ACTAAGGGGA CTCAGAGGCT	60
GGTGGGATCC TCCATATGCT GAACGTTGGT TCCCGGGGCC CCCTTATTTT TTTCTCTATA	120
CTTTGTCTCT GTGTCTTTTT CTTTTC AAG TCTTCTTCAT TTGCACCTTA CGAGAAACAT	180
CTCCATCATG GTTGTGGAT GGGGGCAA	208

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1060 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..1060
- (D) OTHER INFORMATION: /note= "FIGURE 7B"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CTGCAGGTGT ACCCAACAGC TCCGAAGAGA CAGTGACATC GAGAACGGGC CATGATGACG	60
ATGGCGGTTT TGTGAAAAG AAAAGGGGA AATGTGGGA AAAGCAAGAG AGATGAGATT	120
GTTACTGTGT CTGTATAGAA AGAAGTAGAC ATAGGAGACT CCATTTTGT CTGTACTAAG	180
AAAAATTCTT CTGCCTTGAG ATGCTGTAA TCTATGACCT TACCCCAAC CCCGTGCTCT	240
CTGAAACATG TGCCGTGTCA AACTCAGGT TAAATGGATT AAGGGTGGTG CAAGATGTGC	300
TTTGTAAAC AGATGCTTGA AGGCAGCATG CTCATTAAGA GTCATCACCA CTCCCTAATC	360
TCAAGTACCC AGGGACACAA AACTGCGAA AGGCCGAGG GACCTCTGCC TAGGAAAGCC	420
AGGTATTGTC CAAGGTTTCT CCCCATGTGA TAGTCTGAAA TATGGCCTCG TGGGAAGGA	480
AAGACCTGAC CATCCCCAG ACCAACACCC GTAAAGGGTC TGTGCTGAGG AGGATTAGTA	540
TAAGAGGAAA GCATGCCTCT TGCACTTGAG AGAAGAGGAA GACATCTGTC TCCTGCCCAT	600
CCCCTGGGCA ATGGAATGTC TCAGTATAAA ACCCGATTGA ACATTCCATC TACTGAGATA	660
GGGAAAACT GCCTTAGGGC TGGAGGTGGG ACATGTGGGC AGCAATACTG CTTGTAAAG	720
CATTGAGATG TTTATGTGTA TGTATATCTA AAAGCACAGC ACTTGATCCT TTACCTTGTC	780
TATGATGCAA ACACCTTTGT TCACGTGTTT GTCTGCTGAC CCTCTCCCA CTATTGTCTT	840
GTGACCCTGA CACATCTCCC TCAGGAGAAA CACCCAGAA TGATCAATAA ATACTAAGGG	900

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GACTCAGAGG CTGGTGGGAT CCTCCATATG CTGAACGTTG GTTCCCGGGG CCCCCTTATT 960
TCTTTCTCTA TACTTTGTCT CTGTGTCTTT TTCTTTTCCA AGTCTTCTTC ATTTGCACCT 1020
TACGAGAAAC ATCTCCATCA TGGTTGTTGG ATGGGGGCAA 1060

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1754 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..1754
- (D) OTHER INFORMATION: /note= "FIGURE 7C"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ATGGTAACAC CAGTCACATG GATGGATAAT CCTATAGAAG TATATGTTAA TGATAGTGTA 60
TGGGTACCTG GCCCCACAGA TGATCGCTGC CTGCCAAAC CTGAGGAAGA AGGGATGATG 120
ATAAATATTT CCATTGGGTA TCATTATCCT CCTATTTGCC TAGGGAGAGC ACCAGGATGT 180
TTAATGCCTG CAGTCCAAAA TTGGTTGGTA GAAGTACCTA CTGTCAGTCC TAACAGTAGA 240
TTCACTTATC ACATGGTAAG CGGGATGTCA CTCAGGCCAC GGGTAAATTA TTTACAAGAC 300
TTTTCTTATC AAAGATCATT AAAATTTAGA CCTAAAGGGA AAACCTGCCC CAAGGAAATT 360
CCTAAAGGAT CAAAGAATAC AGAAGTTTTA GTTTGGGAAG AATGTGTGGC CAATAGTGTG 420
GTGATATTAC AAAACAATGA ATTCGGAAC ATTATAGATT AGGCACCTCG AGGTCAATTC 480
TACCACAATT GCTCAGGACA AACTCAGTCG TGTCCAAGTG CACAAGTGAG TCCAGCTGTC 540
GATAGCGACT TAACAGAAAG TCTAGACAAA CATAAGCATA AAAAATTACA GTCTTTCTAC 600
CTTTGGGAAT GGAAGAAAA AGGAATCTCT ACCCCAAGAC CAAAAATAAT AAGTCCTGTT 660
TCTGGTCCTG AACATCCAGA ATTGTGGAGG CTTACTGTGG CCTCACACCA CATTAGAATT 720
TGGTCTGGAA ATCAACITT AGAAACAAGA TATCGTAAGC CATTTTATAC TATCGACCTA 780
AATTCCATTC TAACGGTTCC TTTACAAAGT TGCCTAAAGC CCCCTTATAT GCTAGTTGTA 840
GGAAATATAG TTATTAAACC AGCCTCCCAA ACTATAACCT GTGAAAATTG TAGATTGTTT 900
ACTTGCATTG ATTCAACTTT TAATTGGCAG CACCGTATTC TGCTGGTGAG AGCAAGAGAA 960
GGCATGTGGA TCCCTGTGTC CACGGACCGA CCGTGGGAGG CCTCGCCATC CATCCATATT 1020

TTGACTGAAA	TATTAAGG	CGTTTTAAAT	AGATCCAAAA	GATTCATTTT	TACTTTAATT	1180
GCAGTGATTA	TGGGATTAAT	TGCAGTCACA	GCTACGGCTG	CTGTGGCAGG	GGTTGCATTG	1190
CACTCTTCTG	TTCAGTCAGT	AAACTTTGTT	AATTATTGGC	AAAAGAATTC	TACAAGATTG	1200
TGGAATTCAC	AATCTAGTAT	TGATCAAAAA	TTGGCAAGTC	AAATTAATGA	TCTTAGACAA	1210
ACTGTCATTT	GGATGGGAGA	CAGGCTTGAC	TTAGAACATC	ATTTCCAGTT	ACAGTGTGAC	1220
TGGAATACGT	CAGATTTTTG	TATTACACCC	CAAATTTATA	ATGAGTCTGA	GCATCACTGG	1230
GACATGGTTA	GACGCCATCT	ACAGGGAAGA	GAAGATAATC	TCACTTTAGA	CATTTCCAAA	1240
TTAAAAGAAC	AAATTTTTCGA	AGCATCAAAA	GCCCATTTAA	ATTTGGTGCC	AGGAACTGAG	1250
GCAATTGCAG	GAGTTGCTGA	TGGCCTCGCA	AATCTTAACC	CTGTCACTTG	GATTAAGACC	1260
ATCAGAAGTA	CTATGATTAT	AAATCTCATA	TTAATCGTTG	TGTGCCTGTT	TTGTCTGTTG	1270
TTAGTCTGCA	GGTGTACCCC	AACAGCTCCG	AAAAAAACAG	TGACATCGAG	AACGGGCCAT	1280
GAATGACAAA	GGCGGTTTTT	GTTCCAAAAA	AAAAAGGGGG	AAATTTTGGG	GAAAACCAAA	1290
AAAATGAAAA	TGTT					1300

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) **FEATURE:**

- (A) NAME/KEY: misc feature
(B) LOCATION:1..520
(D) OTHER INFORMATION:/note= "FIGURE 7D"

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 59..517

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

ACATTTGAAG TTCTACAATG AACCCATCAG AGATGCAAAG AAAAGCGCCT CCACGGAG	58
ATG GTA ACA CCA GTC ACA TGG ATG GAT AAT CCT ATA GAA GTA TAT GTT	106
Met Val Thr Pro Val Thr Trp Met Asp Asn Pro Ile Glu Val Tyr Val	
1 5 10 15	
AAT GAT AGT GTA TGG GTA CCT GGC CCC ACA GAT GAT CGC TGC CCT GCC	154
Asn Asp Ser Val Trp Val Pro Gly Pro Thr Asp Asp Arg Cys Pro Ala	
20 25 30	

AAA CCT GAG GAA GAA GGG ATG ATG ATA AAT ATT TCC ATT GGG TAT CAT	202
Lys Pro Glu Glu Glu Gly Met Met Ile Asn Ile Ser Ile Gly Tyr His	
35 40 45	
TAT CCT CCT ATT TGC CTA GGG AGA GCA CCA GGA TGT TTA ATG CCT GCA	250
Tyr Pro Pro Ile Cys Leu Gly Arg Ala Pro Gly Cys Leu Met Pro Ala	
50 55 60	
GTC CAA AAT TGG TTG GTA GAA GTA CCT ACT GTC AGT CCT AAC AGT AGA	298
Val Gln Asn Trp Leu Val Glu Val Pro Thr Val Ser Pro Asn Ser Arg	
65 70 75 80	
TTC ACT TAT CAC ATG GTA AGC GGG ATG TCA CTC AGG CCA CGG GTA AAT	346
Phe Thr Tyr His Met Val Ser Gly Met Ser Leu Arg Pro Arg Val Asn	
85 90 95	
TAT TTA CAA GAC TTT TCT TAT CAA AGA TCA TTA AAA TTT AGA CCT AAA	394
Tyr Leu Gln Asp Phe Ser Tyr Gln Arg Ser Leu Lys Phe Arg Pro Lys	
100 105 110	
GGG AAA ACT TGC CCC AAG GAA ATT CCT AAA GGA TCA AAG AAT ACA GAA	442
Gly Lys Thr Cys Pro Lys Glu Ile Pro Lys Gly Ser Lys Asn Thr Glu	
115 120 125	
GTT TTA GTT TGG GAA GAA TGT GTG GCC AAT AGT GTG GTG ATA TTA CAA	490
Val Leu Val Trp Glu Glu Cys Val Ala Asn Ser Val Val Ile Leu Gln	
130 135 140	
AAC AAT GAA TTC GGA ACT ATT ATA GAT TAG	520
Asn Asn Glu Phe Gly Thr Ile Ile Asp	
145 150	

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 153 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Met Val Thr Pro Val Thr Trp Met Asp Asn Pro Ile Glu Val Tyr Val	
1 5 10 15	
Asn Asp Ser Val Trp Val Pro Gly Pro Thr Asp Asp Arg Cys Pro Ala	
20 25 30	
Lys Pro Glu Glu Glu Gly Met Met Ile Asn Ile Ser Ile Gly Tyr His	
35 40 45	
Tyr Pro Pro Ile Cys Leu Gly Arg Ala Pro Gly Cys Leu Met Pro Ala	
50 55 60	
Val Gln Asn Trp Leu Val Glu Val Pro Thr Val Ser Pro Asn Ser Arg	
65 70 75 80	
Phe Thr Tyr His Met Val Ser Gly Met Ser Leu Arg Pro Arg Val Asn	
85 90 95	

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Tyr Leu Gln Asp Phe Ser Tyr Gln Arg Ser Leu Lys Phe Arg Pro Lys
 100 105 110
 Gly Lys Thr Cys Pro Lys Glu Ile Pro Lys Gly Ser Lys Asn Thr Glu
 115 120 125
 Val Leu Val Trp Glu Glu Cys Val Ala Asn Ser Val Val Ile Leu Gln
 130 135 140
 Asn Asn Glu Phe Gly Thr Ile Ile Asp
 145 150

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 603 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "retroviral DNA"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..603
- (D) OTHER INFORMATION: /note= "FIGURE 7E"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ACATTTGAAG TTCTACAATG AACCCATCAG AGATGCAAAG AAAAGCGCCT CCACGGAGAT	60
GGTAACACCA GTCACATGGA TGGATAATCC TATAGAAGTA TATGTTAATG ATAGTGTATG	120
GGTACCTGGC CCCACAGATG ATCGCTGCCC TGCCAAACCT GAGGAAGAAG GGATGATGAT	180
AAATATTTCC ATTGGGTATC ATTATCCTCC TATTTGCCTA GGGAGAGCAC CAGGATGTTT	240
AATGCCTGCA GTCCAAAATT GGTGGTAGA AGTACCTACT GTCAGTCCTA ACAGTAGATT	300
CACTTATCAC ATGGTAAGCG GGATGTCACT CAGGCCACGG GTAAATTATT TACAAGACTT	360
TTCTTATCAA AGATCATTAA AATTTAGACC TAAAGGGAAA ACTTGCCCCA AGGAAATTCC	420
TAAAGGATCA AAGAATACAG AAGTTTtagT TTGGGAAGAA TGTGTGGCCA ATAGTGTGGT	480
GATATTACAA AACAATGAAT TCGGAACAT TATAGATTAG GCACCTCGAG GTCAATTCTA	540
CCACAATTGC TCAGGACAAA CTCAGTCGTG TCCAAGTGCA CAAGTGAGTC CAGCTGTCGA	600
TAG	603

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(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 561 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION:1..561
- (D) OTHER INFORMATION:/note= "Figure 7F"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```

Met Val Thr Pro Val Thr Trp Met Asp Asn Pro Ile Glu Val Tyr Val
1           5           10           15

Asn Asp Ser Val Trp Val Pro Gly Pro Thr Asp Asp Arg Cys Pro Ala
          20           25           30

Lys Pro Glu Glu Gly Met Met Ile Asn Ile Ser Ile Gly Tyr His
          35           40           45

Tyr Pro Pro Ile Cys Leu Gly Arg Ala Pro Gly Cys Leu Met Pro Ala
          50           55           60

Val Gln Asn Trp Leu Val Glu Val Pro Thr Val Ser Pro Asn Ser Arg
65           70           75           80

Phe Thr Tyr His Met Val Ser Gly Met Ser Leu Arg Pro Arg Val Asn
          85           90           95

Tyr Leu Gln Asp Phe Ser Tyr Gln Arg Ser Leu Lys Phe Arg Pro Lys
          100          105          110

Gly Lys Thr Cys Pro Lys Glu Ile Pro Lys Gly Ser Lys Asn Thr Glu
          115          120          125

Val Leu Val Trp Glu Glu Cys Val Ala Asn Ser Val Val Ile Leu Gln
          130          135          140

Asn Asn Glu Phe Gly Thr Ile Ile Asp Glx Ala Pro Arg Gly Gln Phe
145          150          155          160

Tyr His Asn Cys Ser Gly Gln Thr Gln Ser Cys Pro Ser Ala Gln Val
          165          170          175

Ser Pro Ala Val Asp Ser Asp Leu Thr Glu Ser Leu Asp Lys His Lys
          180          185          190

His Lys Lys Leu Gln Ser Phe Tyr Leu Trp Glu Trp Glu Glu Lys Gly
          195          200          205

Ile Ser Thr Pro Arg Pro Lys Ile Ile Ser Pro Val Ser Gly Pro Glu
          210          215          220

His Pro Glu Leu Trp Arg Leu Thr Val Ala Ser His His Ile Arg Ile
225          230          235          240

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Trp Ser Gly Asn Gln Thr Leu Glu Thr Arg Tyr Arg Lys Pro Phe Tyr
 245 250 255
 Thr Ile Asp Leu Asn Ser Ile Leu Thr Val Pro Leu Gln Ser Cys Leu
 260 265 270
 Lys Pro Pro Tyr Met Leu Val Val Gly Asn Ile Val Ile Lys Pro Ala
 275 280 285
 Ser Gln Thr Ile Thr Cys Glu Asn Cys Arg Leu Phe Thr Cys Ile Asp
 290 295 300
 Ser Thr Phe Asn Trp Gln His Arg Ile Leu Leu Val Arg Ala Arg Glu
 305 310 315 320
 Gly Met Trp Ile Pro Val Ser Thr Asp Arg Pro Trp Glu Ala Ser Pro
 325 330 335
 Ser Ile His Ile Leu Thr Glu Ile Leu Lys Gly Val Leu Asn Arg Ser
 340 345 350
 Lys Arg Phe Ile Phe Thr Leu Ile Ala Val Ile Met Gly Leu Ile Ala
 355 360 365
 Val Thr Ala Thr Ala Ala Val Ala Gly Val Ala Leu His Ser Ser Val
 370 375 380
 Gln Ser Val Asn Phe Val Asn Tyr Trp Gln Lys Asn Ser Thr Arg Leu
 385 390 395 400
 Trp Asn Ser Gln Ser Ser Ile Asp Gln Lys Leu Ala Ser Gln Ile Asn
 405 410 415
 Asp Leu Arg Gln Thr Val Ile Trp Met Gly Asp Arg Leu Asp Leu Glu
 420 425 430
 His His Phe Gln Leu Gln Cys Asp Trp Asn Thr Ser Asp Phe Cys Ile
 435 440 445
 Thr Pro Gln Ile Tyr Asn Glu Ser Glu His His Trp Asp Met Val Arg
 450 455 460
 Arg His Leu Gln Gly Arg Glu Asp Asn Leu Thr Leu Asp Ile Ser Lys
 465 470 475 480
 Leu Lys Glu Gln Ile Phe Glu Ala Ser Lys Ala His Leu Asn Leu Val
 485 490 495
 Pro Gly Thr Glu Ala Ile Ala Gly Val Ala Asp Gly Leu Ala Asn Leu
 500 505 510
 Asn Pro Val Thr Trp Ile Lys Thr Ile Arg Ser Thr Met Ile Ile Asn
 515 520 525
 Leu Ile Leu Ile Val Val Cys Leu Phe Cys Leu Leu Leu Val Cys Arg
 530 535 540
 Cys Thr Pro Thr Ala Pro Lys Lys Thr Val Thr Ser Arg Thr Gly His
 545 550 555 560
 Glu

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(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 604 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..604
- (D) OTHER INFORMATION:/note= "FIGURE 7G"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:59..601

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

ACATTTGAAG TTCTACAATG AACCCATCAG AGATGCAAAG AAAAGCGCCT CCACGGAG	58
ATG GTA ACA CCA GTC ACA TGG ATG GAT AAT CCT ATA GAA GTA TAT GTT	106
Met Val Thr Pro Val Thr Trp Met Asp Asn Pro Ile Glu Val Tyr Val	
1 5 10 15	
AAT GAT AGT GTA TGG GTA CCT GGC CCC ACA GAT GAT CGC TGC CCT GCC	154
Asn Asp Ser Val Trp Val Pro Gly Pro Thr Asp Asp Arg Cys Pro Ala	
20 25 30	
AAA CCT GAG GAA GAA GGG ATG ATG ATA AAT ATT TCC ATT GGG TAT CAT	202
Lys Pro Glu Glu Glu Gly Met Met Ile Asn Ile Ser Ile Gly Tyr His	
35 40 45	
TAT CCT CCT ATT TGC CTA GGG AGA GCA CCA GGA TGT TTA ATG CCT GCA	250
Tyr Pro Pro Ile Cys Leu Gly Arg Ala Pro Gly Cys Leu Met Pro Ala	
50 55 60	
GTC CAA AAT TGG TTG GTA GAA GTA CCT ACT GTC AGT CCT AAC AGT AGA	298
Val Gln Asn Trp Leu Val Glu Val Pro Thr Val Ser Pro Asn Ser Arg	
65 70 75 80	
TTC ACT TAT CAC ATG GTA AGC GGG ATG TCA CTC AGG CCA CGG GTA AAT	346
Phe Thr Tyr His Met Val Ser Gly Met Ser Leu Arg Pro Arg Val Asn	
85 90 95	
TAT TTA CAA GAC TTT TCT TAT CAA AGA TCA TTA AAA TTT AGA CCT AAA	394
Tyr Leu Gln Asp Phe Ser Tyr Gln Arg Ser Leu Lys Phe Arg Pro Lys	
100 105 110	
GGG AAA ACT TGC CCC AAG GAA ATT CCT AAA GGA TCA AAG AAT ACA GAA	442
Gly Lys Thr Cys Pro Lys Glu Ile Pro Lys Gly Ser Lys Asn Thr Glu	
115 120 125	
GTT TTA GTT TGG GAA GAA TGT GTG GCC AAT AGT GTG GTG ATA TTA CAA	490
Val Leu Val Trp Glu Glu Cys Val Ala Asn Ser Val Val Ile Leu Gln	
130 135 140	

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AAC AAT GAA TTC GGA ACT ATT ATA GAT TTA GGC ACC TCG AGG TCA ATT 538
 Asn Asn Glu Phe Gly Thr Ile Ile Asp Leu Gly Thr Ser Arg Ser Ile
 145 150 155 160
 CTA CCA CAA TTG CTC AGG ACA AAC TCA GTC GTG TCC AAG TGC ACA AGT 586
 Leu Pro Gln Leu Leu Arg Thr Asn Ser Val Val Ser Lys Cys Thr Ser
 165 170 175
 GAG TCC AGC TGT CGA TAG 604
 Glu Ser Ser Cys Arg
 180

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Met Val Thr Pro Val Thr Trp Met Asp Asn Pro Ile Glu Val Tyr Val
 1 5 10 15
 Asn Asp Ser Val Trp Val Pro Gly Pro Thr Asp Asp Arg Cys Pro Ala
 20 25 30
 Lys Pro Glu Glu Gly Met Met Ile Asn Ile Ser Ile Gly Tyr His
 35 40 45
 Tyr Pro Pro Ile Cys Leu Gly Arg Ala Pro Gly Cys Leu Met Pro Ala
 50 55 60
 Val Gln Asn Trp Leu Val Glu Val Pro Thr Val Ser Pro Asn Ser Arg
 65 70 75 80
 Phe Thr Tyr His Met Val Ser Gly Met Ser Leu Arg Pro Arg Val Asn
 85 90 95
 Tyr Leu Gln Asp Phe Ser Tyr Gln Arg Ser Leu Lys Phe Arg Pro Lys
 100 105 110
 Gly Lys Thr Cys Pro Lys Glu Ile Pro Lys Gly Ser Lys Asn Thr Glu
 115 120 125
 Val Leu Val Trp Glu Glu Cys Val Ala Asn Ser Val Val Ile Leu Gln
 130 135 140
 Asn Asn Glu Phe Gly Thr Ile Ile Asp Leu Gly Thr Ser Arg Ser Ile
 145 150 155 160
 Leu Pro Gln Leu Leu Arg Thr Asn Ser Val Val Ser Lys Cys Thr Ser
 165 170 175
 Glu Ser Ser Cys Arg
 180

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..182
- (D) OTHER INFORMATION: /note= "FIGURE 7H"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```

Phe Thr Ile Pro Leu Ala Glu Gln Asp Cys Glu Lys Phe Ala Phe Thr
1           5           10           15

Ile Pro Ala Ile Asn Asn Lys Glu Pro Ala Thr Arg Phe Gln Trp Lys
20           25           30

Val Leu Pro Gln Gly Met Leu Asn Ser Pro Thr Ile Cys Gln Thr Phe
35           40           45

Val Gly Arg Ala Leu Gln Pro Val Arg Asp Lys Phe Ser Asp Cys Tyr
50           55           60

Ile Ile His Tyr Phe Asp Asp Ile Leu Cys Ala Ala Glu Thr Lys Asp
65           70           75           80

Lys Leu Ile Asp Cys Tyr Thr Phe Leu Pro Ala Glu Val Ala Asn Ala
85           90           95

Gly Leu Ala Ile Ala Ser Asp Lys Ile Gln Thr Ser Thr Pro Phe His
100          105          110

Tyr Leu Gly Met Gln Ile Glu Asn Arg Lys Ile Lys Pro Gln Lys Ile
115          120          125

Glu Ile Arg Lys Asp Thr Leu Lys Thr Leu Asn Asp Phe Gln Lys Leu
130          135          140

Leu Gly Asp Ile Asn Trp Ile Arg Pro Thr Leu Gly Ile Pro Thr Tyr
145          150          155          160

Ala Met Ser Asn Leu Phe Ser Ile Leu Arg Gly Asp Ser Asp Leu Asn
165          170          175

Ser Lys Arg Met Leu Thr
180

```

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 250 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "retroviral DNA"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION:1..250

(D) OTHER INFORMATION:/note= "FIGURE 8A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GTAAATGACA CCTATGATGC ACTGCCACCC TTTCACGTGT TCACCCTGAA CATCTGCTTT	60
TTACATCTAA GTGATTGTAC CCAATAAATA GTGTGGAGAC CAGAGCTCTG AGCCTTTTGC	120
AGCCTCCATT TTGCAACTGG TCCCCTGGCT CCCACCTTTA TGAACCTTTA ACCTGTCTTT	180
TCTCATTCCT TTGTCACCAT TGGACTTTGG GTACCCTACG GGTGGTGTG AGGCTGTCAC	240
CGCACATTAA	250

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 203 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION:1..203

(D) OTHER INFORMATION:/note= "FIGURE 8B"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GTTTAGTTAA TCTATAATCT ATAGAGACAA TGCTTATCAC TGGCTTGCTG TCAATAAATA	60
TGTGGGTAAA TCTCTGTCA AGACTCTCAG CTTTGAAGCT GTGAGACCCC TGATTTCCTA	120
CTCCACACCT CTATATTCT GTGTGTGTGT CTTTAATTCC TCCAGTGTG CTGGGTTAGG	180
GTCTCCTCGA CGAGCTGTCG TGC	203

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 283 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "RETROVIRAL DNA"

- (ix) FEATURE:
(A) NAME/KEY: misc feature
(B) LOCATION:1..283
(D) OTHER INFORMATION:/note= "FIGURE 8C"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

AAC	TCA	GCTG	CTG	CACAGT	G	GTC	GAGCCTC	CAGAGCTCAT	GCCATTGCAG	TGGTCAGAGC	60
CTG	GCCCTCC	TCTTCCTGCA	TAGAACCTGG	ATTCAATCTG	TAAGGTGGGA	AGTGCAGCAG	120				
CAGAGAACTC	TGGCCTTGCA	GAGAGTCCCT	GTTCCCACTT	CAC	TTTCCTT	TTCACCAAAT	180				
AAAACCCTGC	TTTCACTCAT	GCATCAAATT	GTCTGTGAGC	CTACATTTTT	GTGGCCATGG	240					
GACAAGAACA	CCATCTTTAG	CTGAGCTAGG	GAAAAGTCCT	GCA		283					

(2) INFORMATION FOR SEQ ID NO: 43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 245 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "retroviral DNA"

- (ix) FEATURE:
(A) NAME/KEY: misc feature
(B) LOCATION:1..245
(D) OTHER INFORMATION:/note= "FIGURE 8D"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GATGTACCA	CTGTGACCTA	CCTACACTGG	AGATGGCTCA	CAC	TTCTTA	CCCTCCCT	60
GCTGTACCAA	TAAATAACAG	CACAGCCTGA	CATTCGGAGC	CATTACCGGT	CTTTGTGACT	120	
TGGTGGTAGT	GGTATCCCCT	AGGGCCCAGC	TGTCTTTTCT	TTTATCTCTT	TGTCTGTGT	180	
CTTTATTCT	ATGAGTCTCT	CGTCTCCGCA	CATGGGGAGA	AAAACCCATA	GACCTGTAG	240	
GGCTG						245	

(2) INFORMATION FOR SEQ ID NO: 44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "retroviral DNA"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION:1..181

(D) OTHER INFORMATION:/note= "FIGURE 8E"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

CTCACAAAAA TAATAAAGC TTCTGTTGGC CATTCTTCAG ATCTTCATCT CTTGTGAGGA	60
TCCCCCTGTA CATGTAAAAA TGAATAAAA CTTGTATCCT TTCTCCTCTT AATCTGTCTT	120
GCATCAATAT CATTCCTAGA CCCAGTCAGA GATGGGTGGA GGTGAGCCGT ACATTTCCT	180
A	181

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 287 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION:1..287

(D) OTHER INFORMATION:/note= "FIGURE 8F"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

CAGAGAACTC CAGCCAGCTG TGATGGAGCC TCAGGAAGTT CACAGTTGCA GCAGGAAGGA	60
GCCTGGCTGC TCCTCTTCCT GTGTGGAACC TGGGATTAGA ACAGGCTGGC AGGAAGTGCT	120
TTAGCAGGGA CTCTGGCCTA CTCACACTCC TTGTTTCCCC CTTTCTTCC TTTTCACTCA	180
ATAAAGCCCT GTCTTACTCA CCATTCAAAT TGTCTGTGAG CCTGAATTTT CATGGCTGTG	240
GGACAAAGAA CCCTATTTTT AGCTGAACTA AGGAAAATTC CTGCAAA	287

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 264 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "RETROVIRAL DNA"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 1..264

(D) OTHER INFORMATION: /note= "FIGURE 8G"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GTGATTGTCT GCTGACCCTC TCCCCACAAT TGTCTTGTGA CCCTGACACA TCCCCCTCTT	60
CGAGAAACAC CCGCGGATGA TCAATAAATA TTAAGGGAAC TCAGAGGCTG GCAGGATCCT	120
CCATATGCTG AACGCTGGTT GCCCCGGGTC CCCTTCTTTC TTTCTCTATA CTTTGTCTCT	180
GTGTCTTTTT CTTTCCAAA TCTCTCGTCC CACCTTACGA GAAACACCCA CAGGTGTGTC	240
CGGGCAACCC AACGCCACAT AACA	264

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